WEST Search History

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DATE: Monday, August 30, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	fsh.clm. same first.clm. same second.clm.	10
☐	L2	L1 and polyclonal and monoclonal	4
☐	L3	antifsh or anti-fsh	37
☐	L4	alpha same beta same (fsh or follicle)	845
☐	L5	L4 same two	142
☐	L6	L5 and sandwich	32

END OF SEARCH HISTORY

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DATE: Monday, August 30, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	fsh.clm. same first.clm. same second.clm.	10
☐	L2	L1 and polyclonal and monoclonal	4
☐	L3	antifsh or anti-fsh	37

END OF SEARCH HISTORY

First Hit Fwd Refs

L6: Entry 20 of 32 File: USPT Apr 16, 1996

DOCUMENT-IDENTIFIER: US 5508261 A
TITLE: Analogs of glycoprotein hormones having altered receptor binding specificity and activity and methods for preparing and using same

Drawing Description Text (7):
FIG. 6 illustrates the half lives of hCG isolated from the urine of pregnant women (uhCG), hCG made in cell culture (rhCG), hFSH made in cell culture (rhFSH), DG (CF94-117) made in cell culture, and PRM1 (CFC94-114) made in cell culture. In these experiments, 5-15 micrograms of the proteins were injected intravenously into the external jugular vein of rats that had been anesthetized with ether. At the times indicated on the abscissa, the rats were reanesthetized and 0.2 ml of blood withdrawn and allowed to clot. The serum from this blood was obtained by centrifuging the clotted blood at 2000.times.g for 30 minutes. For measurements of hCG, DG, and PRM1, aliquots of the serum (0.1-50 microliters) were used in sandwich immunoassays employing A113 as a capture antibody and radioiodinated B105 as a detection antibody. hCG purified from urine was used as a standard curve to measure hCG, DG, and PRM1. For measurements of hFSH, aliquots of the serum (0.1-50 microliters) were used in sandwich immunoassays employing A113 as a capture antibody antibody and radioiodinated B602 as detection antibody. hFSH purified from tissue culture medium was used as a standard to measure hFSH. FIG. 6 illustrates that all the analogs can be detected in serum for several hours after they have been injected into rats. The value obtained at 1 hour was normalized to be 100% and the amounts of materials that remained after this time were expressed as a percentage of the amount at 1 hour. This method permits the relative stability of the materials to be seen. Urinary hCG, rhCG, and PRM1 are the most stable compounds and could be detected 24 or more hours after injection. The vertical bar in FIG. 6 extends to the upper limit of the standard error of the mean.

Detailed Description Text (3):
The alpha, beta-heterodimeric polypeptides of the present invention are "engineered" "engineered" to alter the LH and FSH receptor binding activity and specificity of the polypeptides in vertebrates. Applicants have found that 1) the region of the hCG beta-subunit between 94-97 (i.e., "D" region) is most important for LH receptor binding activity and specificity, 2) the region of the hFSH beta-subunit between 100-106 (hCG numbering, i.e., "G" region) is most important for FSH binding activity and specificity and, 3) these regions ("D" and "G") of the beta-subunit are somewhat independent in activity. Substitution of a non-LH sequence in the "D" region will decrease binding of the polypeptide to the LH receptor. Substitution of a FSH sequence in the "G" region will increase binding of the polypeptide to the FSH receptor. Thus, it is possible to make modifications and substitutions in these regions to decrease the affinity of hCG for LH receptors and to dramatically increase the affinity of hCG for FSH receptors. The region of the beta-subunit between 1-93 and the choice of alpha-subunit does not appear to be important for LH and FSH binding activity and specificity. Hence analogs having a wide variety of activities can be made by altering the compositions of these two critical regions.

Detailed Description Text (24):
These values were obtained by dividing the concentration of analog required to inhibit .sup.125 I-hCG binding to rat luteal ovarian LH receptors or .sup.125 I-hFSH binding to bovine testes FSH receptors by 50% into that required for recombinant hCG and recombinant hFSH, respectively. The concentrations of the

analogs were determined by sandwich immunoassay using antibodies B105 and All3. The term "N.D." means not determined because concentrations of analog were not employed high enough to detect 50% inhibition. This was because the amounts of hFSH needed to bind to LH Receptors are several orders of magnitude greater than that of hCG and vice versa the amounts of hCG needed to bind to FSH receptors are several orders of magnitude greater than that of hFSH.

Detailed Description Text (46):
An aliquot of the ligation mixture was taken and used to transform DH5-alpha strain E. coli. (obtained from Bethesda Research Laboratories, Gaithersburg, Md.). Plasmid DNAs from ampicillin-resistant DH5-alpha clones were screened by digestion with BglII (which is unique to vectors containing the cassette) and EcoRI (which cuts in the vector). Positive clones were identified by the presence of two fragments (approximately 0.8 Kbp and 2.9 Kbp). The sequence in the coding region of one of these plasmids, which lacked most of the beta-subunit cDNA due to excision of the PvuII fragment, was confirmed by dideoxysequencing as described (10). The remainder of the beta-subunit cDNA (encoding hCG.beta. amino acids 1-87) was restored by ligation of the 2.3 Kbp PvuI-PvuII fragment of this vector and the 2.9 Kbp PvuI-PvuII fragment from pSVL-hCG-beta'. The ligation mixture was used to transform DH5-alpha strain E. coli. and ampicillin resistant clones were obtained. Miniprep plasmid DNA from these clones were digested with EcoRI and BglII, and DNA from positive clones exhibited fragments of approximately 2.5 Kbp and 2.9 Kbp. After the DNA was subjected to a dideoxy sequencing procedure to confirm that it encoded "GT" (Table 1), the plasmid DNA was then cotransfected into COS-7 cells (obtained from the American Type Culture Collection) along with pSVL-hCG-alpha, a pSVL-based plasmid encoding the human glycoprotein hormone alpha-subunit (10, 21), using a DEAE-dextran procedure (10). Beginning in 1-2 days and for a few days thereafter, the COS-7 cells produced significant amounts of the free subunits and the heterodimer. These were present in the culture media and heterodimer was detected using sandwich immunoassays employing monoclonal antibodies All3 and B105 (10). The protein was concentrated by ultrafiltration and monitored for its abilities to bind to LH and FSH receptors by radioligand receptor assays using .sup.125 I-hCG and .sup.125 I-hFSH as tracers and rat ovarian corpora lutea and bovine testes as sources of LH and FSH receptors as described (10).

Detailed Description Text (48):
We have found that an alpha, beta-heterodimer composed of the alpha-subunit of hCG and an hCG/hFSH beta-subunit chimera termed "G" having the amino acid sequence illustrated in Table 1 has high affinity for LH and FSH receptors as shown by its ability to compete with radiolabeled hCG and/or hFSH for binding to these receptors (Table 3, FIGS. 1 and 2). This analog can be prepared in a variety of methods well-known to one versed in the art of molecular biology, one of which is described here. The cDNA for analog "GT' was digested with BglII and SstI and the 5.2-5.3 Kbp fragment was ligated with the oligonucleotides: ##STR4## using standard methods (23, 24). The ligation mixture was used to transform competent DH5-alpha strain E. coli. (23, 24). Transformed cells were selected by their abilities to grow on agar plates containing amphicillin. Ampicillin resistant colonies were chosen and plasmid minipreparations were made by the boiling lysis method (23, 24). The plasmid DNA was then tested for the presence of HindIII-ApaI endonuclease restriction sites. Plasmid DNA having the desired sequences was cleaved into three fragments (approximately 0.8 Kbp, 1.1 Kbp, and 3.4 Kbp). After the DNA was subjected to a dideoxy sequencing procedure to confirm that it encoded "G" (Table 1), the plasmid DNA was then cotransfected into COS-7 cells (obtained from the American Type Culture Collection) along with pSVL-hCG-alpha, a pSVL-based plasmid encoding the alpha-subunit (10, 21), using a DEAE-dextran procedure (10, 21, 23, 24). Beginning in 1-2 days and for a few days thereafter, the COS-7 cells produced significant amounts of the free subunits and the heterodimer. These were present in the culture media and heterodimer was detected using sandwich immunoassays employing monoclonal antibodies A113 and B105 (10). The protein was concentrated by ultrafiltration and monitored for its abilities to bind to LH and FSH receptors by

radioligand receptor assays using .sup.125 I-hCG and .sup.125 I-hFSH as tracers and rat ovarian corpora lutea and bovine testes as sources of LH and FSH receptors as described (10).

Detailed Description Text (50):
Preparations of analogs "D" and "DG'" have been described previously (10) and are the same as those of analogs CF94-97 and CF94-114 in that report, respectively. Analog "Q" was prepared from the expression vector encoding analogs "D" and CF108-114 (10) by digesting them with PpuMI, separating the fragments on agarose gels, and ligating the large fragment obtained from CF108-114 with the small fragment obtained from "D." The resulting plasmid was then cotransfected into COS-7 cells along with pSVL-hCG-alpha and the media assayed for the presence of the analogs using an A113-B105 sandwich immunoassay as described (10).

Detailed Description Text (53):
The ligation mixture was used to transform DH5A E. coli. and miniprep plasmid DNA obtained from ampicillin resistant colonies was screened for the presence of an approximately 0.6Kbp fragment released by digestion with AccI. After DNA sequencing was performed to confirm that the construct encoded the desired sequence, it was cut with PvuII and ligated with the 1.6 Kbp fragment of pSVL-hCG.beta.'. The ligation product was transformed into DH5-alpha strain E. coli. and positive clones were selected. Plasmid DNA was prepared by boiling lysis and digested with EcoNI and XhoI. DNA which had the insert in the correct orientation produced fragments approximately 2.6 Kbp, 1.7 Kbp, 0.5 Kbp, 0.25 Kbp, and 0.15 Kbp. The plasmid DNA was then cotransfected into COS-7 cells (obtained from the American Type Culture Collection) along with pSVL-hCG-alpha, a pSVL-based plasmid encoding the alpha-subunit (10, 21), using a DEAE-dextran procedure (10, 21, 23, 24). Beginning in 1-2 days and for a few days thereafter, the COS-7 cells produced significant amounts of the free subunits and the heterodimer. These were present in the culture media and heterodimer was detected using sandwich immunoassays employing monoclonal antibodies antibodies A113 and B105 (10). The concentration of the protein was concentrated by ultrafiltration and monitored for its abilities to bind to LH and FSH receptors by radioligand receptor assays using .sup.125 I-hCG and .sup.125 I-hFSH as tracers and rat ovarian corpora lutea and bovine testes as sources of LH and FSH receptors as described (10).

Detailed Description Text (60):
As illustrated in FIG. 6, the half-life of DG is approximately the same as that of hFSH and less than that of urinary hCG. The half-life of PRM1 appears to be slightly longer. These half-live studies were performed by injecting approximately 5-15 .mu.g of hormones i.v. into a rat and then drawing samples at various times (indicated on the figure). The amount of material in serum was estimated by sandwich immunoassay employing antibodies A113 for capture and radioiodinated B105 for detection of hCG, DG, G, and PRM1. For hFSH we used a sandwich assay based on A113 and radioiodinated B602. These assays have been described in other patent applications.

10201089 PMID: 8085741

[Current data on the structure-activity relationship of gonadotropins]

Donnees recentes sur les relations structure-activite des hormones gonadotropes.

Bidart J M

Service d'Immunologie Moleculaire, Institut Gustave-Roussy, Villejuif.

Annales pharmaceutiques francaises (FRANCE) 1994, 52 (1) p1-10,
ISSN 0003-4509 Journal Code: 2985176R

Document type: Journal Article; Review; Review, Tutorial ; English Abstract

Languages: FRENCH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Most of the available information on the structure-function of gonadotropins is critical for the development of both therapeutic approaches to reproductive malfunctions and new strategies for the control of fertility. Major advances in our understanding of the structure of these hormones and the manner in which they interact with their receptors have been made in recent years. Furthermore, the recent availability of highly purified hormones and their recombinant counterparts as well as the cloning of he receptors offer new tools for the investigation of structure-activity relationship of gonadotropins. The present article **reviews** : (i) the overall structure of these complex glycoprotein hormones assembled from two distinct **alpha** (**alpha**) and **beta** (**beta**) subunits, (ii) their heterogeneities in term of carbohydrate and peptidic structures, which influence both the bioactivity and the immunoreactivity of the hormones, (iii) the structure and the functional domains of their receptors, belonging to the G-protein coupled receptor family, and (iv) the more recent information available on the regions of interaction between the subunits and between the hormone and its corresponding receptor. Pioneering work on the topographical features of gonadotropins has been accomplished in a piecemeal fashion by using classic chemical methods of protein modification. In the last ten years, new approaches, including the production of monoclonal antibodies, the construction of synthetic peptides and the use of recombinant DNA technology, provided important insights in the knowledge of these molecules. However, significant progress remains to be accomplished, particularly in the establishment of the three-dimensional structure of these hormones, which are critical for the understanding of pathophysiological processes related to endocrinology and for the design of new peptidic or nonpeptidic analogs useful of therapeutic approaches. (42 Refs.)

Tags: Human

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service.  Enter a BEGIN command plus a file number to search a database
(e.g., B1 for ERIC).
?b 155

       30aug04 16:49:45 User228206 Session D2223.1
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     $0.00  Estimated cost FileHomeBase
     $0.00  Estimated cost this search
     $0.00  Estimated total session cost   0.160 DialUnits


File 155:MEDLINE(R) 1951-2004/Aug W5
       (c) format only 2004 The Dialog Corp.
*File 155: Medline has been reloaded. Accession numbers
have changed. Please see HELP NEWS 154 for details.

      Set  Items  Description
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?s review? or tutor?
          550270  REVIEW?
            2539  TUTOR?
      S1  552502  REVIEW? OR TUTOR?
?s (fsh? or (follicle (2n) stimulat?))
           21893  FSH?
           46164  FOLLICLE
          708673  STIMULAT?
           30308  FOLLICLE(2N)STIMULAT?
      S2   35318  (FSH? OR (FOLLICLE (2N) STIMULAT?))
?s s1 and s2
          552502  S1
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         2446177  PY=2000 : PY=2004
      S4     240  S3/2000:2004
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          499005  BETA?
      S6      19  S5 AND ALPHA? AND BETA?
?t s6/9/all
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9/6,KWIC/47
DIALOG(R)File 155:(c) format only 2004 The Dialog Corp. All rts. reserv.

13849968 PMID: 9550532
The effects of menopausal status and exercise training on serum lipids and the activities of intravascular enzymes related to lipid transport.
Apr 1998

... untrained eumenorrheic, premenopausal (PRM) women (n = 21; mean age, 36 +/- 3 years) and estrogen-free **postmenopausal** (POM) women (n = 16; mean age, 68 +/- 8 years). Subjects trained at a progressive intensity...

...weeks on a standardized diet designed to maintain body weight and during the early follicular **stage** for the PRM group. Blood samples were analyzed for serum **total** cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), the cholesterol content of the...

... apo)A-I and apoB, lipoprotein(a), and the activity of lecithin:cholesterol acyltransferase (LCAT). **Total** and hepatic triglyceride lipase activity (HTGLA) were determined from plasma samples obtained after heparin administration...
Descriptors: Lipase--blood--BL; *Lipids--blood--BL; *Lipoprotein Lipase --blood--BL; * **Menopause** --blood--BL; *Phosphatidylcholine-Sterol O-Acyltransferase--blood--BL; *Physical Education and Training...; Analysis of Variance; Apolipoproteins--blood--BL; Biological Transport; Endothelium, Vascular--enzymology--EN; Lipids--pharmacokinetics--PK; **Postmenopause** --blood--BL; Premenopause--blood--BL

9/6,KWIC/48
DIALOG(R)File 155:(c) format only 2004 The Dialog Corp. All rts. reserv.

13820436 PMID: 9518873
Development, validation and application of an ultra-sensitive two-site enzyme immunoassay for human follistatin.
Feb 1998

... 288. The presence of sodium deoxycholate and Tween 20 in the diluent gave results for **total** (free and activin-dissociated) follistatin. The assay had a detection limit of <19 pg/ml...

...showed fluctuating follistatin concentrations (approximately 0.62 ng/ml) with no apparent relationship to the **stage** of the cycle. Interestingly, pooled serum from **postmenopausal** women appeared to have higher follistatin levels than any of the normal women (approximately 1...
...; blood--BL; Granulosa Cells--secretion--SE; Isomerism; Menstruation --blood--BL; Mice; Mice, Inbred BALB C; **Postmenopause** --blood--BL; Regression Analysis; Semen--chemistry--CH; Sensitivity and Specificity

9/6,KWIC/49
DIALOG(R)File 155:(c) format only 2004 The Dialog Corp. All rts. reserv.

13797206 PMID: 9494212
[Health education on menopause: consensus on its contents]
Educacion para la salud sobre el climaterio: un consenso sobre sus contenidos.
Dec 1997

...define the standard knowledge women should have about the menopause in order to confront this **stage** successfully. DESIGN: Use of the Delphi technique to reach consensus with experts at a national...

... psychological and social health fields, who had professional experience in women's problems at this **stage** of their lives, and/or authors of books, articles, doctoral theses and other documents on...

08959698 PMID: 1906350

Development of a luminescence immunoassay **for** follitropin **suitable for clinical routine.**

Biro G; Samira A; Butz H; Leicht E; Weinges K F

Medizinische Klinik und Poliklinik, Innere Medizin II, Universitat des Saarlandes, Germany.

European journal of clinical chemistry and clinical biochemistry - journal of the Forum of European Clinical Chemistry Societies (GERMANY) Mar 1991, 29 (3) p189-92, ISSN 0939-4974 Journal Code: 9105775

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We developed a luminescence **immunoassay** (LIA) for **follitropin** , based on the synthesis of a **follitropin** -N-(4-aminobutyl)-N-ethylisoluminol conjugate. The luminescence tracer was purified by gel chromatography. Antibody-bound and non-bound tracer fractions were separated by using a second antibody reagent bound to magnetic particles. The assay can be performed within 24 hours and is sufficiently sensitive for the measurement of all clinically relevant follitropin concentrations including the subnormal range.

Tags: Human

Descriptors: *Follicle Stimulating Hormone--analysis--AN; Analysis of Variance; Diagnostic Tests, Routine--methods--MT; Immunoassay--methods--MT; Indicators and Reagents; Luminescence

CAS Registry No.: 0 (Indicators and Reagents); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19910816

Record Date Completed: 19910816

09228842 PMID: 1547548

Multianalyte immunoassay based on spatially distinct fluorescent areas quantified by laser-excited solid-phase time-resolved fluorometry.

Kakabakos S E; Christopoulos T K; Diamandis E P

Department of Clinical Biochemistry, Toronto Western Hospital, Ontario, Canada.

Clinical chemistry (UNITED STATES) Mar 1992, 38 (3) p338-42, ISSN 0009-9147 Journal Code: 9421549

Comment in Clin Chem. 1992 Mar;38(3) 327-8; Comment in PMID 1547545

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We describe a new multianalyte **immunoassay** principle and apply it to the simultaneous **immunoassay** of lutropin, **follitropin** , choriogonadotropin, and prolactin in serum. The method is based on the coating of distinct areas of polystyrene with analyte-specific antibodies. These antibodies react with the analyte and immobilize it in a specific area while another biotinylated antibody also reacts with the analyte to form a sandwich. After addition of streptavidin labeled with the fluorescent europium chelate of 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid, fluorescent areas are formed, the intensity of which is related to the amount of each analyte present in the sample. The fluorescent areas are quantified on the dry solid phase with laser-excited time-resolved fluorometric measurements. The assays developed are highly sensitive, precise, and accurate. We believe that this system shows potential for multianalyte immunoassay of diverse groups of compounds in disciplines such as endocrinology, infectious disease, hematology, and oncology.

Tags: Comparative Study; Human; Support, Non-U.S. Gov't

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File 155:MEDLINE(R) 1951-2004/Aug W4
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have changed. Please see HELP NEWS 154 for details.

      Set  Items  Description
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?e gonadotrop

Ref   Items  Index-term
E1        1  GONADOTRONAIA
E2        2  GONADOTROOP
E3       33 *GONADOTROP
E4       36  GONADOTROPA
E5        1  GONADOTROPAN
E6        1  GONADOTROPAS
E7      734  GONADOTROPE
E8       42  GONADOTROPEN
E9       31  GONADOTROPER
E10     821  GONADOTROPES
E11       1  GONADOTROPESPREHYPOPHYSAIRES
E12     619  GONADOTROPH

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?s e8 or e3
              42  GONADOTROPEN
              33  GONADOTROP
      S1      75  'GONADOTROPEN' OR 'GONADOTROP'
?e hormone

Ref   Items  Index-term
E1        3  HORMONDRUSEN
E2        1  HORMONDRUSENUBERFUNKTION
E3   252357 *HORMONE
E4     7558  HORMONE   //CORTICOTROPIN-RELEASING   (CORTICOTROPIN-RELEASING
             HORMONE)
E5    26976  HORMONE //FOLLICLE STIMULATING (FOLLICLE STIMULATING HORMONE)
E6    34918  HORMONE //GROWTH (GROWTH HORMONE)
E7     5574  HORMONE //HUMAN GROWTH (HUMAN GROWTH HORMONE)
E8    36917  HORMONE //LUTEINIZING (LUTEINIZING HORMONE)
E9      478  HORMONE   //MSH   RELEASE-INHIBITING  (MSH  RELEASE-INHIBITING
             HORMONE)
E10      26  HORMONE //MSH-RELEASING (MSH-RELEASING HORMONE)
E11   17507  HORMONE //PARATHYROID (PARATHYROID HORMONE)
E12     136  HORMONE      //PROLACTIN     RELEASE-INHIBITING     (PROLACTIN
             RELEASE-INHIBITING HORMONE)

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?s e3
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Ref   Items   RT  Index-term
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                  HORMONE)
E14     934       HORMONE  //RECEPTORS, CORTICOTROPIN-RELEASING (RECEPTORS,
                  CORTICOTROPIN-RELEASING HORMONE)
E15    1021       HORMONE    //RECEPTORS,    GASTROINTESTINAL   (RECEPTORS,
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E16       4       HORMONE  //RECEPTORS,  PANCREATIC  (RECEPTORS, PANCREATIC
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                HORMONE)
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                HORMONE)
E19    2696     HORMONE //RECEPTORS, THYROID (RECEPTORS, THYROID HORMONE)
E20     480     HORMONE  //RECEPTORS,  THYROTROPIN-RELEASING  (RECEPTORS,
                THYROTROPIN-RELEASING HORMONE)
E21    4003     HORMONE  //SOMATOTROPIN-RELEASING (SOMATOTROPIN-RELEASING
                HORMONE)
E22  690324     HORMONE ANT //HORMONES, HORMONE SUBSTITUTES, A (HORMONES,
                HORMONE SUBSTITUTES, AND HORMONE ANT)
E23    3072  43 HORMONE ANTAGONISTS
E24     252     HORMONE ANTAGONISTS --ADMINISTRATION AND DOSAG

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E25     123  HORMONE ANTAGONISTS --ADVERSE EFFECTS --AE
E26       1  HORMONE ANTAGONISTS --AGONISTS --AG
E27      24  HORMONE ANTAGONISTS --ANALYSIS --AN
E28       1  HORMONE ANTAGONISTS --BIOSYNTHESIS --BI
E29      31  HORMONE ANTAGONISTS --BLOOD --BL
E30      28  HORMONE ANTAGONISTS --CHEMICAL SYNTHESIS --CS
E31      77  HORMONE ANTAGONISTS --CHEMISTRY --CH
E32       1  HORMONE ANTAGONISTS --CLASSIFICATION --CL
E33       1  HORMONE ANTAGONISTS --CONTRAINDICATIONS --CT
E34      19  HORMONE ANTAGONISTS --DIAGNOSTIC USE --DU
E35       4  HORMONE ANTAGONISTS --ECONOMICS --EC
E36       3  HORMONE ANTAGONISTS --HISTORY --HI

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Ref   Items  Index-term
E37      16  HORMONE ANTAGONISTS --IMMUNOLOGY --IM
E38      21  HORMONE ANTAGONISTS --ISOLATION AND PURIFICATI
E39     132  HORMONE ANTAGONISTS --METABOLISM --ME
E40      54  HORMONE ANTAGONISTS --PHARMACOKINETICS --PK
E41    1696  HORMONE ANTAGONISTS --PHARMACOLOGY --PD
E42       6  HORMONE ANTAGONISTS --PHYSIOLOGY --PH
E43       1  HORMONE ANTAGONISTS --SECRETION --SE
E44     454  HORMONE ANTAGONISTS --THERAPEUTIC USE --TU
E45      53  HORMONE ANTAGONISTS --TOXICITY --TO
E46       8  HORMONE ANTAGONISTS --URINE --UR
E47      31  HORMONE ANTAGONISTS--ADMINISTRACTION AND DOSAG
E48       7  HORMONE ANTAGONISTS--ANALYSIS

          Enter P or PAGE for more
?p

Ref   Items  Index-term
E49       1  HORMONE ANTAGONISTS--MEN
E50       5  HORMONE ANTAGONISTS--PHARMACODYNAMICS
?p

Ref   Items    RT  Index-term
E1        5       HORMONE ANTAGONISTS--PHARMACODYNAMICS
E2       17       HORMONE ANTAGONISTS--SIDE EFFECTS
E3       10       HORMONE ANTAGONISTS--THERAPEUTIC USE
E4        1       HORMONE RECEPTOR 39, DROSOPHILA
E5      303       HORMONE RECEPTORS
E6       48       HORMONE   RECEPTORS   ALPHA  //THYROID  (THYROID  HORMONE
                  RECEPTORS ALPHA)
E7       43       HORMONE   RECEPTORS   BETA   //THYROID  (THYROID  HORMONE
                  RECEPTORS BETA)
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E8        82        HORMONE RECEPTORS--ANALYSIS
E9         1        HORMONE RECEPTORS--PHARMACODYNAMICS
E10        3        HORMONE RECEPTORS--THERAPEUTIC USE
E11        0     1  HORMONE RECEPTORS, CELL SURFACE
E12        0     1  HORMONE RECEPTORS, CYTOPLASMIC

          Enter P or PAGE for more
?p

Ref    Items    RT  Index-term
E13        0     1  HORMONE RECEPTORS, NUCLEAR
E14       88        HORMONE RELEASE INHIBITING HORMONES //PITUITAR (PITUITARY
                    HORMONE RELEASE INHIBITING HORMONES)
E15     2860     3  HORMONE REPLACEMENT THERAPY
E16      652        HORMONE REPLACEMENT THERAPY --ADVERSE EFFECTS
E17        2        HORMONE REPLACEMENT THERAPY --CLASSIFICATION -
E18       46        HORMONE REPLACEMENT THERAPY --CONTRAINDICATION
E19       30        HORMONE REPLACEMENT THERAPY --ECONOMICS --EC
E20        4        HORMONE REPLACEMENT THERAPY --HISTORY --HI
E21      268        HORMONE REPLACEMENT THERAPY --METHODS --MT
E22        5        HORMONE REPLACEMENT THERAPY --MORTALITY --MO
E23        5        HORMONE REPLACEMENT THERAPY --NURSING --NU
E24       55        HORMONE REPLACEMENT THERAPY --PSYCHOLOGY --PX

          Enter P or PAGE for more
?p

Ref    Items    RT  Index-term
E25       24        HORMONE REPLACEMENT THERAPY --STANDARDS --ST
E26       57        HORMONE REPLACEMENT THERAPY --STATISTICS AND N
E27       32        HORMONE REPLACEMENT THERAPY --TRENDS --TD
E28       71        HORMONE REPLACEMENT THERAPY --UTILIZATION --UT
E29        1        HORMONE REPLACEMENT THERAPY--BENEFICIAL EFFECT
E30        0     1  HORMONE REPLACEMENT THERAPY, POST-MENOPAUSAL
E31      165        HORMONE RESISTANCE SYNDROME //THYROID (THYROID HORMONE
                    RESISTANCE SYNDROME)
E32   690324        HORMONE SUBSTITUTES, AND HORMONE AN//HORMONES, (HORMONES,
                    HORMONE SUBSTITUTES, AND HORMONE ANT)
E33     3187        HORMONE-BINDING GLOBULIN //SEX (SEX HORMONE-BINDING
                    GLOBULIN)
E34     3139        HORMONE-DEPENDENT //NEOPLASMS, (NEOPLASMS, HORMONE-DEPEN-
                    DENT)
E35        0     1  HORMONE-DEPENDENT NEOPLASMS
E36      230        HORMONE-REGULATING HORMON //RECEPTORS, PITUITA
                    (RECEPTORS, PITUITARY HORMONE-REGULATING HORMON)

          Enter P or PAGE for more
?p

Ref    Items    Index-term
E37     1951    HORMONE-RELATED PROTEIN //PARATHYROID (PARATHYROID
                HORMONE-RELATED PROTEIN)
E38     3074    HORMONE-RELEASING HORMONES //PITUITARY (PITUITARY
                HORMONE-RELEASING HORMONES)
E39      347    HORMONE, BETA SUBUNIT //FOLLICLE STIMULATING (FOLLICLE
                STIMULATING HORMONE, BETA SUBUNIT)
E40       60    HORMONE, BETA SUBUNIT //LUTEINIZING (LUTEINIZING HORMONE, BETA
                SUBUNIT)
E41       90    HORMONE, HUMAN //FOLLICLE STIMULATING (FOLLICLE STIMULATING
                HORMONE, HUMAN)
E42      359    HORMONE, TYPE 1 //RECEPTOR, PARATHYROID (RECEPTOR, PARATHYROID
                HORMONE, TYPE 1)
E43       29    HORMONE, TYPE 2 //RECEPTOR, PARATHYROID (RECEPTOR, PARATHYROID
                HORMONE, TYPE 2)
E44        1    HORMONEACTIVE
E45        1    HORMONEACTIVITY
E46        2    HORMONEAL
```

```
E47         1  HORMONEANALYTIC
E48         1  HORMONEAUX
           Enter P or PAGE for more
?p

Ref    Items  Index-term
E49         5  HORMONEBINDING
E50         3  HORMONECH
?p

Ref    Items  Index-term
E1          3  HORMONECH
E2          1  HORMONECOMPETENT
E3          1  HORMONECONCENTRATIONS
E4          1  HORMONECONTAINING
E5          1  HORMONED
E6          1  HORMONEDEPENDENCE
E7          2  HORMONEDEPENDENCY
E8          6  HORMONEDEPENDENT
E9          1  HORMONEE
E10         1  HORMONEFFEKT
E11         2  HORMONEFFEKTE
E12         1  HORMONEFFEKTER

           Enter P or PAGE for more
?p

Ref    Items  Index-term
E13         1  HORMONEIGF
E14         2  HORMONEIHIN
E15         1  HORMONEINDEPENDENT
E16         5  HORMONEINFLUSS
E17         3  HORMONEINNAHME
E18         1  HORMONEINPFLANZUNG
E19         1  HORMONEINWIRKUNG
E20         1  HORMONEINWIRKUNGEN
E21         3  HORMONEISTA
E22         1  HORMONEJASMONATE
E23         5  HORMONEL
E24         1  HORMONELA

           Enter P or PAGE for more
?e gonadotropin

Ref    Items  Index-term
E1          2  GONADOTROPIINIRESEPTORIT
E2          1  GONADOTROPIINIT
E3      39931 *GONADOTROPIN
E4      21336  GONADOTROPIN //CHORIONIC (CHORIONIC GONADOTROPIN)
E5        233  GONADOTROPIN //RECEPTORS, (RECEPTORS, GONADOTROPIN)
E6          2  GONADOTROPIN I BETA SUBUNIT, FUNDULUS
E7          5  GONADOTROPIN I BETA-SUBUNIT, BASS
E8          2  GONADOTROPIN I, KATSUWONUS
E9          1  GONADOTROPIN I, TUNA
E10         3  GONADOTROPIN II ALPHA SUBUNIT, CATFISH
E11         4  GONADOTROPIN II BETA SUBUNIT, BASS
E12         6  GONADOTROPIN II BETA SUBUNIT, CATFISH

           Enter P or PAGE for more
?s gonadotropin?
      S3   53477  GONADOTROPIN?
?p

Ref    Items   RT  Index-term
E13         2       GONADOTROPIN II BETA SUBUNIT, FUNDULUS
E14         3       GONADOTROPIN II, KATSUWONUS
E15         1       GONADOTROPIN II, TUNA
```

```
E16       7       GONADOTROPIN INHIBITOR
E17       0    1  GONADOTROPIN RECEPTORS
E18      57       GONADOTROPIN RELEASING HORMONE ASSOCIATED PEPT
E19       0    1  GONADOTROPIN RELEASING-HORMONE RECEPTORS
E20      32       GONADOTROPIN- PITUITARY, BETA-SUBUNIT I, SALMO
E21       5       GONADOTROPIN-ASSOCIATED-PEPTIDE RELEASING ENZY
E22       5       GONADOTROPIN-INHIBITORY HORMONE
E23       3       GONADOTROPIN-REGULATED TESTICULAR RNA HELICASE
E24       0    1  GONADOTROPIN-RELEASING HORMONE

          Enter P or PAGE for more
?p

Ref    Items   RT  Index-term
E25        0    1  GONADOTROPIN-RESISTANT OVARY SYNDROME
E26     2181       GONADOTROPIN,  BETA SUBUNIT, HUMAN //CHORIONIC (CHORIONIC
                   GONADOTROPIN, BETA SUBUNIT, HUMAN)
E27       48       GONADOTROPIN, PITUITARY, BETA-SUBUNIT II
E28      362       GONADOTROPINA
E29        1       GONADOTROPINABSONDERUNG
E30        1       GONADOTROPINAKTIVITAT
E31        2       GONADOTROPINAM
E32        8       GONADOTROPINAMI
E33        1       GONADOTROPINANALYSER
E34        1       GONADOTROPINANTWORT
E35        1       GONADOTROPINAPPLIKATION
E36        1       GONADOTROPINAPPLIKATIONEN

          Enter P or PAGE for more
?p

Ref    Items  Index-term
E37       59  GONADOTROPINAS
E38        1  GONADOTROPINAUSCHUTTUNG
E39        7  GONADOTROPINAUSSCHEIDUNG
E40        2  GONADOTROPINAUSSCHUTTUNG
E41        1  GONADOTROPINAUSSEHEIDUNG
E42        1  GONADOTROPINAUSTESTUNG
E43        2  GONADOTROPINBEHANDLING
E44        8  GONADOTROPINBEHANDLUNG
E45        1  GONADOTROPINBEHANDLUNGSERFOLGE
E46        2  GONADOTROPINBESTIMMUNG
E47        1  GONADOTROPINBESTIMMUNGEN
E48        2  GONADOTROPINDS

          Enter P or PAGE for more
?p

Ref    Items  Index-term
E49      287  GONADOTROPINE
E50        5  GONADOTROPINEM
?ds

Set     Items   Description
S1         75   'GONADOTROPEN' OR 'GONADOTROP'
S2     252357   'HORMONE'
S3      53477   GONADOTROPIN?
?s (s1 or s2 or s3) and perimeno? and premenopa? and postmenopa?
              75  S1
          252357  S2
           53477  S3
            1661  PERIMENO?
            8884  PREMENOPA?
           25829  POSTMENOPA?
      S4     134  (S1 OR S2 OR S3) AND PERIMENO? AND PREMENOPA? AND
                  POSTMENOPA?
?s s4/2000:2004
```

```
             134  S4
         2437013  PY=2000 : PY=2004
      S5      50  S4/2000:2004
?s s4 not s5
             134  S4
              50  S5
      S6      84  S4 NOT S5
?s s6 and (antibod? or assay? or immunoassay? or monoclonal? or differenti? or diagno?
or determ? or measur? or test?)
              84  S6
          666463  ANTIBOD?
          472390  ASSAY?
           39433  IMMUNOASSAY?
          176656  MONOCLONAL?
          701254  DIFFERENTI?
         1942873  DIAGNO?
         1351608  DETERM?
         1216307  MEASUR?
         1561418  TEST?
      S7      63  S6 AND (ANTIBOD? OR ASSAY? OR IMMUNOASSAY? OR MONOCLONAL?
                  OR DIFFERENTI? OR DIAGNO? OR DETERM? OR MEASUR? OR TEST?)
?t s7/6/all
```

7/6/1
16663877 PMID: 15304903
Quantitative bone ultrasonometry in climacteric women.
Jan 1998

7/6/2
16492170 PMID: 15251741
Can follicle-stimulating hormone **be used to define menopausal status?**
1998

7/6/3
14523988 PMID: 10524483
Abnormal uterine bleeding.
Oct 1 1999

7/6/4
14522764 PMID: 10522991
Plasma cholesterol esterification and transfer, the menopause, and hormone **replacement therapy in women.**
Oct 1999

7/6/5
14515870 PMID: 10515677
Hormone **replacement therapy in** perimenopausal **women and 2-year change of carotid intima-media thickness.**
Aug 16 1999

7/6/6
14379114 PMID: 10374217
Symptom responses of midlife Filipina Americans.
Summer 1999

7/6/7
14377590 PMID: 10372280
Depressive symptoms in the perimenopause **: prevalence, assessment, and guidelines for treatment.**
Sep-Oct 1998

```
     $0.99  TELNET
    $10.42  Estimated cost this search
    $10.68  Estimated total session cost   3.027 DialUnits


### Status: Signed Off. (4 minutes)

### Status: Path 1 of [Dialog Information Services via Modem]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)
Trying 31060000009998...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS?
### Status: Signing onto Dialog
 ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS? ********
Welcome to DIALOG
### Status: Connected


Dialog level 04.11.00D

Reconnected in file 155 24aug04 16:57:42
* * * *

File 155:MEDLINE(R) 1951-2004/Aug W4
       (c) format only 2004 The Dialog Corp.
*File 155: Medline has been reloaded. Accession numbers
have changed. Please see HELP NEWS 154 for details.

      Set  Items  Description
      ---  -----  -----------
Cost is in DialUnits
?ds

Set     Items   Description
S1         75   'GONADOTROPEN' OR 'GONADOTROP'
S2     252357   'HORMONE'
S3      53477   GONADOTROPIN?
S4        134   (S1 OR S2 OR S3) AND PERIMENO? AND PREMENOPA? AND POSTMENO-
              PA?
S5         50   S4/2000:2004
S6         84   S4 NOT S5
S7         63   S6 AND (ANTIBOD? OR ASSAY? OR IMMUNOASSAY? OR MONOCLONAL? -
              OR DIFFERENTI? OR DIAGNO? OR DETERM? OR MEASUR? OR TEST?)
?t s7/9/2 56 44 47 49 12 13 14 15 20 21 25 27 29 31 34 40 41 54 58 63

 7/9/2
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

16492170   PMID: 15251741
  Can follicle-stimulating hormone be used to define menopausal status?
  Stellato SM R K; Crawford PhD S L; McKinlay PhD S M; Longcope MD C
  New England Research Institutes, Watertown, Massachusetts.
  Endocrine practice - official journal of the American College of
Endocrinology and the American Association of Clinical Endocrinologists (
United States) 1998, 4 (3) p137-41, ISSN 1530-891X Journal Code:
9607439
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: In Data Review
```

Objective: To assess the ability of the level of follicle-stimulating **hormone** (FSH) to distinguish among **premenopausal** , **perimenopausal** , and **postmenopausal** women. Methods: We examined cross-sectional and longitudinal data from the second phase of the Massachusetts Women's Health Study (1986 to 1995), a population-based cohort of 427 **premenopausal** and **perimenopausal** women identified from the first phase of the Massachusetts Women's Health Study (1981 to 1986). Results: Boxplots of FSH levels throughout the menopausal transition displayed considerable overlap. Logistic regressions and their resulting receiver operating characteristic curves further demonstrated that, although FSH is a statistically significant predictor of menopausal status, no single value of FSH is expedient for distinguishing **premenopausal** from **perimenopausal** or **perimenopausal** from **postmenopausal** women. Conclusion: FSH alone is not an effective predictor of transition into the **perimenopausal** or **postmenopausal** period. Specifically, the frequently recommended FSH cutoff of 40 IU/L is inappropriate by itself for clinical **determination** of **postmenopausal** status.

Record Date Created: 20040714

7/9/56

DIALOG(R)File 155:MEDLINE(R)

08603776 PMID: 2375337

Relationship between plasma calcium fractions, other bone-related variables, and serum follicle-stimulating hormone **levels in** premenopausal , perimenopausal , **and** postmenopausal **women.**

Nordin B E; Morris H A; Need A G; Horowitz M; Robertson W G

Division of Clinical Chemistry, Institute of Medical and Veterinary Science, Adelaide, Australia.

American journal of obstetrics and gynecology (UNITED STATES) Jul 1990, 163 (1 Pt 1) p140-5, ISSN 0002-9378 Journal Code: 0370476

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

The study comprises 186 untreated normal **premenopausal** , **perimenopausal** , and **postmenopausal** women in whom we **measured** serum follicle-stimulating **hormone** and a number of bone-related plasma and urinary variables. The calcium fractions in the plasma were calculated from the total calcium, albumin, globulin, anion gap, and bicarbonate concentrations. With a level of follicle-stimulating **hormone** within the reference range (up to 20 U/L) to define the **premenopausal** state, we confirmed previously reported menopausal rises in plasma calcium, phosphate, alkaline phosphatase, and bicarbonate, and in urinary calcium and hydroxyproline. However, inspection of the data, and t **testing** at different follicle-stimulating **hormone** criteria showed that these changes in bone-related variables did not generally occur until the level of follicle-stimulating **hormone** exceeded approximately 50 U/L. The plasma alkaline phosphatase level rose earlier than the other variables and was significantly elevated in subjects with follicle-stimulating **hormone** values above 30 U/L. The rise in plasma calcium was mainly a result of a rise in the ultrafiltrable fraction, which in turn was accounted for by rises in the ionized and complexed fractions, of which the complexed fraction was the most significant and proportionately the largest. The rise in the complexed fraction was accounted for by the increase in plasma bicarbonate.

Tags: Female; Human

Descriptors: *Bone and Bones--metabolism--ME; *Calcium--blood--BL; *Menopause--blood--BL; Adult; Alkaline Phosphatase--blood--BL; Bicarbonates --blood--BL; Blood Proteins--metabolism--ME; Calcium--metabolism--ME; Chemical Fractionation; Cross-Sectional Studies; Middle Aged

CAS Registry No.: 0 (Bicarbonates); 0 (Blood Proteins); 7440-70-2 (Calcium)

Enzyme No.: EC 3.1.3.1 (Alkaline Phosphatase)

10341356 PMID: 7839330
Endocrinological changes in pre- and postmenopause]
Endokrinologische Veranderungen in der Pra- und der **Postmenopause** .
von Holst T
Abteilung fur Gynakologische Endokrinologie und Fertilitatsstorungen, Universitats-Frauenklinik Heidelberg.
Therapeutische Umschau. Revue therapeutique (SWITZERLAND) Nov 1994, 51 (11) p722-8, ISSN 0040-5930 Journal Code: 0407224
Document type: Journal Article ; English Abstract
Languages: GERMAN
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The endocrinology of the **perimenopause** --the time between pre- and **postmenopause** --is characterized by changes in the metabolism of the steroid hormones caused by increasing insufficiency of the ovaries. Until the age of 48 the concentrations of the estrogens are relatively constant with a median level of 120 pg/ml serum for estradiol and of 75 pg/ml for estrone. Between the age of 49 and 54 the levels decrease to concentrations of 35 pg/ml for estrone and 10 pg/ml for estradiol. In the corresponding time, there is a tenfold rise of the level of FSH. The level remains constant until high age. The decrease of the estrogens causes the menopause in an age of 51 to 52. In the **postmenopause** the ovaries don't play a role for the concentrations of the estrogens. The concentrations are **determined** by the conversion of the androgens secreted by the adrenal cortex. The serum concentrations of androstenedione are five times higher than those of **testosterone** . The function of the adrenal cortex remains until high age; there is no 'adrenopause' comparable to the 'menopause'. The suppression of the adrenal cortex by treatment with corticoids (e.g. for asthma) causes a dramatic decrease of the androgens and consecutively for the estrogens. The lack of estrogens play an important role in the induction of osteoporosis and other disturbances of the late **postmenopause** , e.g. coronary heart disease. Obese women show in the pre- and the **perimenopause** more often dysfunctional bleedings caused by anovulation or corpus luteum insufficiency.(ABSTRACT TRUNCATED AT 250 WORDS)
Tags: Female; Human
Descriptors: Estrogens--metabolism--ME; * **Postmenopause** --metabolism--ME; * **Premenopause** --metabolism--ME; Adult; Aged; Androgens--metabolism--ME; Follicle Stimulating **Hormone** --metabolism--ME; Middle Aged; Obesity --metabolism--ME; Osteoporosis, **Postmenopausal** --metabolism--ME
CAS Registry No.: 0 (Androgens); 0 (Estrogens); 9002-68-0 (Follicle Stimulating Hormone)
Record Date Created: 19950301
Record Date Completed: 19950301

7/9/49
DIALOG(R)File 155:MEDLINE(R)

10173746 PMID: 8062141
Ovarian hormone **status, life-style factors, and markers of bone metabolism in women aged 50 years.**
Leino A; Jarvisalo J; Impivaara O; Kaitsaari M
Social Insurance Institution, Research and Development Unit, Turku, Finland.
Calcified tissue international (UNITED STATES) Apr 1994, 54 (4) p262-7, ISSN 0171-967X Journal Code: 7905481
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Fifty-year-old women (n = 519) attending a health examination were divided by their ovarian **hormone** status into four groups: **premenopausal**

Tags: Female; Human; Support, Non-U.S. Gov't
Descriptors: *Attitude to Health; *Health Status; *Menopause--physiology --PH; *Menopause--psychology--PX; *Questionnaires--standards--ST; *Self Concept; *Women--psychology--PX; Cross-Sectional Studies; Factor Analysis, Statistical; Longitudinal Studies; Middle Aged; Reproducibility of Results; Victoria; Women--education--ED
Record Date Created: 19990218
Record Date Completed: 19990218

7/9/13
DIALOG(R)File 155:MEDLINE(R)

14122759 PMID: 9819784
Relationship between dermato-physiological changes and hormonal status in pre-, peri-, and postmenopausal **women.**
Ohta H; Makita K; Kawashima T; Kinoshita S; Takenouchi M; Nozawa S
Department of Obstetrics and Gynecology, School of Medicine, Keio University, Tokyo, Japan.
Maturitas (IRELAND) Sep 20 1998, 30 (1) p55-62, ISSN 0378-5122 Journal Code: 7807333
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We previously reported that hormonal changes in **perimenopausal** women are associated with dermatologic changes. In the present study, we evaluated such dermatologic changes by means of dermato-physiological **testing** methods in **perimenopausal** women with various types of hormonal conditions. The study group consisted of 46 consecutive women 41-70 years of age (mean, 54.0 years), attending a climacteric outpatient clinic for a healthy check-up is whom no abnormalities were recognized. The women were divided into four groups according to menstrual history and sex-related steroid **hormone** values: a **premenopausal** group (n = 9); a **perimenopausal** group (n = 8); an early menopausal group, in which 5 years or less had elapsed since menopause (n = 12); and a late menopausal group, in which 6 years or more had elapsed since menopause (n = 17). We found that: (1) after menopause the sebum cutaneum content of the forehead decreased significantly, but that of the subocular region was unchanged; (2) the water content of the stratum corneum of the forehead was significantly higher in the late menopausal group than in the **premenopausal** group and the **perimenopausal** group, but there were no significant differences among the four groups at the other sites studied; (3) on psychological stimulation, sweat production was found to decrease significantly after menopause; (4) the skin temperature of the forehead and cheek fell significantly after menopause, but that of the nose, back of the foot, and tips of the toes, did not differ significantly among the four groups; (5) The **perimenopausal** period was associated with increased skin permeability and vascular responsiveness; (6) fingertip plethysmography revealed significant decreases in peripheral circulatory function in the **perimenopausal** group and the late menopausal group.
Tags: Female; Human
Descriptors: *Gonadal Steroid Hormones--blood--BL; *Menopause--physiology --PH; *Skin Physiology; Adult; Aged; Middle Aged; Permeability; **Postmenopause** --physiology--PH; **Premenopause** --physiology--PH; Skin Temperature; Water--metabolism--ME
CAS Registry No.: 0 (Gonadal Steroid Hormones); 7732-18-5 (Water)
Record Date Created: 19990129
Record Date Completed: 19990129

7/9/14
DIALOG(R)File 155:MEDLINE(R)

USA.
Journal of women's health / the official publication of the Society for the Advancement of Women's Health Research (UNITED STATES) Oct 1997, 6 (5) p553-8, ISSN 1059-7115 Journal Code: 9208978
Contract/Grant No.: R01-2R01HL28266; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

We designed a prospective observational trial to study the relationship of thyroid function to cholesterol and weight changes at menopause. Subjects were participants in the ongoing Healthy Women Study, a prospective study of cardiovascular risk factor change through menopause. Healthy **premenopausal** women were recruited from a random sample of licensed drivers in selected ZIP codes of Allegheny County, Pennsylvania. Participants had to be 42-50 years of age, have menstruated within the last 3 months, not have had surgical menopause, have diastolic blood pressure < 100 mm Hg, and not be taking medications (including insulin, estrogen, lipid-lowering drugs, or thyroid or antihypertensive medications) at the baseline examination. The substudy included three groups of women who were **premenopausal** at baseline and were categorized according to change noted at follow-up regarding menopausal status and use of **hormone** replacement therapy (HRT). The groups comprised 95 women who remained **premenopausal**, 96 **postmenopausal** women not on HRT, and 61 **postmenopausal** women using HRT. The main outcome **measures** were baseline and follow-up **measurements** for serum levels of thyroid-stimulating **hormone** (TSH), thyroid peroxidase, and thyroglobulin, as well as serum cholesterol, total high-density lipoprotein (HDL) cholesterol, triglycerides, and calculated low-density lipoprotein (LDL) cholesterol, height, and weight. Covariates included cigarette smoking and alcohol intake. The prevalence of thyroid **antibodies** in this healthy population was high at both time points (range 27%-31%) and did not differ by menopausal status. The presence of thyroid **antibodies** was associated with increased TSH concentration. Women with **antibodies** at both time points had lower levels of total and LDL cholesterol compared with those with no **antibodies**, significant only for those women who remained **premenopausal** during the follow-up period. This healthy population is unlikely to [illegible] lipoprotein and body [illegible] lower

7/9/20
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

13665534 PMID: 9356978
Thyroid function and perimenopausal **lipid and weight changes: the Thyroid Study in Healthy Women (TSH-W).**
Massoudi M S; Meilahn E N; Orchard T J; Foley T P; Kuller L H; Costantino J P; Buhari A M
Graduate School of Public Health, University of Pittsburgh, Pennsylvania,

(Thyrotropin)
Enzyme No.: EC 1.11.1.8 (Iodide Peroxidase)
Record Date Created: 19971126
Record Date Completed: 19971126

7/9/21
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

13568206 PMID: 9255084
Women's health in midlife: the influence of the menopause, social factors and health in earlier life.
Kuh D L; Wadsworth M; Hardy R
MRC National Survey of Health and Development, Department of Epidemiology and Public Health, London, UK.

British journal of obstetrics and gynaecology (ENGLAND) Aug 1997, 104 (8) p923-33, ISSN 0306-5456 Journal Code: 7503752
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: AIM; INDEX MEDICUS

OBJECTIVE: To describe the health symptoms of a large representative sample of British women at age 47 years, and to examine the influence of the menopause allowing for social factors and health in earlier adult life. DESIGN: A national prospective birth cohort study. Information on health problems, menstrual cycle, use of **hormone** replacement therapy and life stress at 47 years was collected using a postal questionnaire. Information on health, smoking behaviour and educational attainment earlier in life had been collected at previous home visits. SETTING: England, Scotland and Wales. POPULATION: A general population sample of 1498 women, 84% of those sent a questionnaire. MAIN OUTCOME **MEASURE** : Twenty self-reported health symptoms over the previous 12 months. RESULTS: Women who had experienced an early natural menopause had a strongly raised risk of vasomotor symptoms (hot flushes or night sweats), sexual difficulties (vaginal dryness or difficulties with intercourse) and trouble sleeping. However, there was little or no excess risk of other somatic or psychological symptoms. In contrast, all types of symptoms were more common among women who had had a hysterectomy or were users of **hormone** replacement therapy. Women with the least education, stressful lives, or a previous history of poor physical and psychological health at age 36 also reported more symptoms at 47 years compared with other women, but adjustment for these factors in a logistic regression model did not affect the relations between symptoms and current menopausal status. For vasomotor symptoms, **postmenopausal** women had an adjusted odds ratio of 4.7 (95% CI 2.6-8.5) and **perimenopausal** women had an adjusted odds ratio of 2.6 (95% CI 1.9-3.5) compared with **premenopausal** women. Corresponding adjusted odds ratios for sexual difficulties were 3.9 (95% CI 2.1-7.1) and 2.2 (95% CI 1.4-3.2), and for trouble sleeping were 3.4 (95% CI 1.9-6.2) and 1.5 (95% CI 1.1-2.0). CONCLUSIONS: Specific symptoms were clearly associated with the natural menopause. More general health concerns were common among women in middle life, particularly among those with stressful lives, or those who had had a hysterectomy or started taking **hormone** replacement therapy before they were **postmenopausal** . Appropriate advice and support needs to be easily accessible.

Tags: Female; Human
Descriptors: *Health Status; *Menopause; *Women's Health; Adult; Age Factors; Attitude to Health; Cohort Studies; Educational Status; Estrogen Replacement Therapy--statistics and numerical data--SN; Great Britain --epidemiology--EP; Hysterectomy--adverse effects--AE; Hysterectomy --statistics and numerical data--SN; Middle Aged; Prospective Studies; Self Disclosure; Sex Disorders--epidemiology--EP; Sleep Disorders--epidemiology --EP; Smoking; Socioeconomic Factors; Stress, Psychological--epidemiology --EP
Record Date Created: 19970904
Record Date Completed: 19970904

7/9/25
DIALOG(R)File 155:MEDLINE(R)

13192818 PMID: 8862496
Bone mineral density and hormone **levels in menopausal Australian women.**
Guthrie J R; Ebeling P R; Hopper J L; Dennerstein L; Wark J D; Burger H G
Key Centre for Women's Health in Society, Department of Public Health and Community Medicine, University of Melbourne, Victoria, Australia.
Gynecological endocrinology - the official journal of the International Society of Gynecological Endocrinology (ENGLAND) Jun 1996, 10 (3) p199-205, ISSN 0951-3590 Journal Code: 8807913
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Subfile: INDEX MEDICUS

To assess the relationships between bone mineral density (BMD) at the lumbar spine and femoral neck and menopausal status, age, physical variables, and lifestyle and gynecological factors. BMD and follicle-stimulating **hormone** (FSH), estradiol and inhibin levels were **measured** in 167 women born in Australia, aged 46-57 years, who had no record of receiving **hormone** replacement therapy. Using the **premenopausal** group as a baseline, the FSH level was higher in peri- and **postmenopausal** subjects ($p < 0.0005$), and estradiol and inhibin levels in the **postmenopausal** women were lower ($p < 0.0005$). Mean (+/- SE) lumbar spine and femoral neck BMD were 15 +/- 3% and 10 +/- 3% lower, respectively, in **postmenopausal** than in **premenopausal** women. Lumbar spine BMD decreased with increasing age in **perimenopausal** women only ($p < 0.005$), and femoral neck BMD decreased with increasing age in the pre-, peri-($p < 0.05$) and **postmenopausal** women. The difference between femoral neck BMD in the pre- and **postmenopausal** women was explained by the difference in age between these groups, whereas for lumbar spine BMD the menopausal status was an additional **determining** factor. There was a negative effect of smoking on femoral neck BMD ($p < 0.05$) in **postmenopausal** women. In the **perimenopausal** decade the femoral neck BMD is primarily dependent on age, whereas lumbar spine BMD is dependent on both age and menopausal status.

Tags: Female; Human; Support, Non-U.S. Gov't

Descriptors: *Bone Density; *Menopause--physiology--PH; Aging; Australia; Estradiol--blood--BL; Femur Neck; Follicle Stimulating **Hormone** --blood--BL ; Inhibins--blood--BL; Lumbar Vertebrae; Luteinizing **Hormone** --blood--BL; Middle Aged; Multivariate Analysis; **Postmenopause** --physiology--PH; Smoking--adverse effects--AE

CAS Registry No.: 50-28-2 (Estradiol); 57285-09-3 (Inhibins); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19961114
Record Date Completed: 19961114

7/9/27

DIALOG(R)File 155:MEDLINE(R)

13142897 PMID: 8810731

Decrease in melatonin precedes follicle-stimulating hormone **increase during** perimenopause .

Vakkuri O; Kivela A; Leppaluoto J; Valtonen M; Kauppila A
Department of Physiology, University of Oulu, Kajaanintie, Finland.

European journal of endocrinology / European Federation of Endocrine Societies (NORWAY) Aug 1996, 135 (2) p188-92, ISSN 0804-4643
Journal Code: 9423848

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Melatonin, the **hormone** of the pineal gland, which in animal studies has been found to inhibit aging processes, is secreted in smaller amounts towards senescence. Menopause, an aging process in women, is known to be associated with typical changes in **gonadotropin** and sex steroid secretion. Our main objective was to study the possible role of melatonin in the hormonal regulation of menopause. This study focused on detailed changes in melatonin and follicle-stimulating **hormone** (FSH) secretion cross-sectionally in pre- to **postmenopausal** females. Special attention was paid to females aged around 50 years, which is the mean menopausal age. Seventy-seven healthy female volunteers aged 30-75 years were the subjects of this study. Melatonin was **measured** radioimmunologically from nocturnal urine collected between 20.00 and 08.00 h, and FSH and melatonin from blood samples taken at 0.900 h. Nocturnal urinary excretion of melatonin was found to decline significantly from **premenopause** to **postmenopause** . The youngest **premenopausal** women (age group 30-39 years) excreted the highest

amounts of melatonin (21.2 +/- 2.2 pmol/h, mean +/- SEM, N = 17). In the age group 40-44 years the excretion declined by 41% ($p < 0.05$). The second significant decline (35%, $p < 0.05$) took place between the age groups 50-54 years and 55-59 years. A declining trend as a function of age was also seen in morning serum melatonin. Serum FSH rose sharply to high levels before the age of 50 ($p < 0.01$) and remained at a high level thereafter. Urinary melatonin correlated negatively with serum FSH ($r = -0.32$, $p < 0.05$). In conclusion, the inverse changes in melatonin and FSH secretion during the **perimenopausal** years, with the sharpest decline in nocturnal excretion of melatonin far before menopause, suggest that melatonin may be permissively linked to the initiation of menopause.

Tags: Female; Human; Support, Non-U.S. Gov't

Descriptors: Melatonin--metabolism--ME; * **Premenopause** --metabolism--ME; Adult; Aged; Aging--blood--BL; Circadian Rhythm; Follicle Stimulating **Hormone** --blood--BL; Melatonin--blood--BL; Melatonin--urine--UR; Middle Aged; **Premenopause** --blood--BL; **Premenopause** --urine--UR

CAS Registry No.: 73-31-4 (Melatonin); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19961204

Record Date Completed: 19961204

7/9/29

DIALOG(R)File 155:MEDLINE(R)

13118191 PMID: 8784098

Bone turnover markers and bone density across the menopausal transition.

Ebeling P R; Atley L M; Guthrie J R; Burger H G; Dennerstein L; Hopper J L; Wark J D

Bone and Mineral Service, University of Melbourne, Royal Melbourne Hospital, Victoria, Australia. p.ebeling@medicine.unimelb.edu.au

Journal of clinical endocrinology and metabolism (UNITED STATES) Sep 1996, 81 (9) p3366-71, ISSN 0021-972X Journal Code: 0375362

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

We **measured** lunbar spine and femoral neck bone mineral density (BMD); urine markers of bone resorption; serum markers of bone formation; and serum gonadotrophin, estradiol and inhibin concentrations in a population-based cohort of 281 women aged 45-57 yr. Women were classified into pre-, peri-, and **postmenopausal** groups, depending on menstrual bleeding patterns. Compared with **premenopausal** women, BMD was lower only in **postmenopausal** women but not in women currently using **hormone** replacement therapy (HRT). BMD decreased with age in the **perimenopausal** group. Compared with **premenopausal** women, **perimenopausal** women had 20% greater urine N-telopeptide excretion ($P < 0.05$) and a doubling of gonadotrophin levels ($P < 0.01$), whereas serum estradiol and bone formation marker concentrations were no different. **Postmenopausal** Women had greater levels of bone turnover markers ($P < 0.0001$), except free deoxypyridinoline and type I procollagen propeptide. Among **postmenopausal** women, bone resorption markers were lower in those using HRT. Levels of nearly all bone turnover markers were positively related to serum FSH concentrations ($P < 0.0001$). Overall, the major independent predictors of BMD were age, urine N-telopeptide, serum bone alkaline phosphatase, and serum, FSH, whereas urine free deoxypyridinoline was positively related to BMD in pre- and **perimenopausal** women. In conclusion, the **perimenopause** is associated with elevated bone resorption rates and declining BMD, and factors in addition to estrogen deficiency may also contribute to the pathogenesis of **postmenopausal** osteoporosis.

Tags: Female; Human; Support, Non-U.S. Gov't

Descriptors: *Biological Markers; *Bone Density; *Bone Remodeling; *Menopause--physiology--PH; Aging; Amino Acids--urine--UR; Bone Resorption; Collagen--urine--UR; Estradiol--blood--BL; Estrogen Replacement Therapy; Femur; Follicle Stimulating **Hormone** --blood--BL; Inhibins--blood--BL;

Luteinizing **Hormone** --blood--BL; Middle Aged; Peptides--urine--UR; **Postmenopause** --physiology--PH; **Premenopause** --physiology--PH; Pyridinium Compounds--urine--UR; Spine
CAS Registry No.: 0 (Amino Acids); 0 (Biological Markers); 0 (Peptides); 0 (Pyridinium Compounds); 0 (collagen type I trimeric cross-linked peptide); 50-28-2 (Estradiol); 57285-09-3 (Inhibins); 63800-01-1 (pyridinoline); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone); 9007-34-5 (Collagen)
Record Date Created: 19961016
Record Date Completed: 19961016

7/9/31
DIALOG(R)File 155:MEDLINE(R)

13042536 PMID: 8708803
Perimenopausal **and** postmenopausal hormone **replacement therapy. Part 2. Hormonal regimens and complementary and alternative therapies.**
Lichtman R
Columbia University School of Nursing, New York, NY 10032, USA.
Journal of nurse-midwifery (UNITED STATES) May-Jun 1996, 41 (3) p195-210, ISSN 0091-2182 Journal Code: 0365647
Document type: Journal Article; Review; Review Literature
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS; NURSING
As midwives increasingly provide care to women throughout the lifecycle, they need to become familiar with a variety of treatments for **perimenopausal** and **postmenopausal** problems and preventive **measures** for the potentially disabling conditions that can accompany aging. This article reviews various regimens of **hormone** replacement therapy. It discusses types of estrogen and progestin, appropriate dosages, routes of administration, and duration of therapy. It offers guidelines for initiation, contraindications, and required follow-up. It addresses the management of side effects and problems. The article briefly reviews a variety of complementary and alternative therapies including various self-help **measures** , such as nutrition and exercise, and selected herbal treatments. Part 1 of this article, which appeared in the January/February 1996 issue of JNM, reviewed the literature on recommended therapeutic and preventive indications for **hormone** replacement therapy, providing guidelines for the provision of informed consent. (109 Refs.)
Tags: Female; Human
Descriptors: Estrogen Replacement Therapy--methods--MT; * **Postmenopause** ; * **Premenopause** ; Complementary Therapies; Estrogen Replacement Therapy --contraindications--CT; Estrogens--therapeutic use--TU; Middle Aged; Practice Guidelines; Progestins--therapeutic use--TU
CAS Registry No.: 0 (Estrogens); 0 (Progestins)
Record Date Created: 19960910
Record Date Completed: 19960910

7/9/34
DIALOG(R)File 155:MEDLINE(R)

12934121 PMID: 8636357
Characterization of reproductive hormonal dynamics in the perimenopause
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Santoro N; Brown J R; Adel T; Skurnick J H
Department of obstetrics and gynecology, University of Medicine and Dentistry of New Jersey, Newark, 07103-2757, USA.
Journal of clinical endocrinology and metabolism (UNITED STATES) Apr 1996, 81 (4) p1495-501, ISSN 0021-972X Journal Code: 0375362
Contract/Grant No.: AG-12222-01; AG; NIA
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: AIM; INDEX MEDICUS

Medical therapy for women in the **perimenopausal** period is controversial, in part due to varying degrees of ovarian **hormone** secretion characteristic of this time of life. To extend our understanding of the reproductive endocrine milieu of **perimenopausal** women, we studied 6 cycling women, aged 47 yr and older, for 6 months with daily collections of first morning voided urine. Five additional older reproductive aged (43-47 yr old) women were studied with daily urine and serum sampling for a single menstrual cycle; their urinary **hormone** data were combined with the former group for menstrual cycle comparisons. Urine was **assayed** for LH, FSH, estrone conjugates, and pregnanediol glucuronide and normalized for creatinine (Cr). Eleven midreproductive aged (19-38 yr old) normally cycling women, 5 women with well defined premature ovarian failure, and 5 women aged 54 yr and older who were at least 1 yr **postmenopausal** were used for comparison. **Perimenopausal** women had shorter follicular phases (11 +/- 2 days vs. 14 +/- 1 days; P = 0.031) and, hence, shorter menstrual cycles than midreproductive aged controls. FSH excretion in **perimenopausal** women was greater than that in younger women (range of means, 4-32 vs 3-7 IU/g Cr; P = 0.0005). LH secretion was overall greater than that in younger normal subjects (range of means, 1.4-6.8 vs. 1.1-4.2 IU/g Cr; P < 0.026). Overall mean estrone conjugate excretion was greater in the **perimenopausal** women compared to that in the younger women [76.9 ng/mg Cr (range, 13.1-135) vs. 40.7 ng/mg Cr (range, 22.8-60.3); P = 0.023] and was similarly elevated in both follicular and luteal phases. Luteal phase pregnanediol excretion was diminished in the **perimenopausal** women compared to that in younger normal subjects (range for integrated pregnanediol, 1.0-8.4 vs. 1.6-12.7 microg/mg Cr/luteal phase; P = 0.015). Compared to **postmenopausal** women, **perimenopausal** women had more overall estrone excretion (2.5-6.2 ng/mg Cr in **postmenopausal** women; P = 0.02) and lower mean FSH (range of means for **postmenopause** , 24-85 IU/g Cr; P = 0.017) and LH (range for **postmenopause** , 4.3-14.8 IU/g Cr; P = 0.041). Compared to women with premature menopause, **perimenopausal** women again had lower FSH (range of means for premature menopause, 36-82 IU/g Cr; P = 0.0022), lower LH (range of means for premature menopause, 5.5-23.8 IU/g Cr; P = 0.0092), borderline higher mean estrone conjugates (range of means for premature menopause, 4-44 ng/mg Cr; P = 0.064), and far longer periods of ovarian activity (one to two cycles in prematurely menopausal women vs. three to six cycles in **perimenopausal** women). We conclude that altered ovarian function in the **perimenopause** can be observed as early as age 43 yr and include hyperestrogenism, hypergonadotropism, and decreased luteal phase progesterone excretion. These hormonal alterations may well be responsible for the increased gynecological morbidity that characterizes this period of life.

Tags: Comparative Study; Female; Human; Support, U.S. Gov't, P.H.S.

Descriptors: Estrone--urine--UR; *Follicle Stimulating **Hormone** --urine --UR; *Luteinizing **Hormone** --urine--UR; *Menstrual Cycle--urine--UR; * **Premenopause** --urine--UR; Adult; Age Factors; Creatinine--urine--UR; Estrogens, Conjugated (USP)--urine--UR; Follicle Stimulating **Hormone** --blood--BL; Follicular Phase; Luteinizing **Hormone** --blood--BL; Menstrual Cycle--blood--BL; Middle Aged; **Premenopause** --blood--BL; Reference Values

CAS Registry No.: 0 (Estrogens, Conjugated (USP)); 53-16-7 (Estrone); 60-27-5 (Creatinine); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19960711
Record Date Completed: 19960711

7/9/40
DIALOG(R)File 155:MEDLINE(R)

12632068 PMID: 7752947

A longitudinal study of the perimenopausal **transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density.**

Rannevik G; Jeppsson S; Johnell O; Bjerre B; Laurell-Borulf Y; Svanberg L
Department of Obstetrics and Gynecology, University of Lund, Malmo General Hospital, Sweden.
Maturitas (IRELAND) Feb 1995, 21 (2) p103-13, ISSN 0378-5122
Journal Code: 7807333
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
From a longitudinal prospective study, 160 women with spontaneous menopause and without steroid medication were followed during the transition from pre- to **postmenopause** . After 12 years 152 women were still participating in the study. Blood samples were drawn every 6 months until 1 year after the menopause and every 12 months thereafter. **Measurements** of bone mineral density (BMD) on the forearm were performed every second year. All women routinely completed a questionnaire concerning symptoms frequently attributed to the climacteric period. All data were grouped around the onset of the menopause, thereby allowing longitudinal evaluation of the changes in the variables from the **premenopausal** to the **postmenopausal** period. The beginning of the **perimenopausal** period was characterized by transitory elevations of follicle-stimulating **hormone** (FSH). A significant increase in serum levels of **gonadotropins** was observed for both FSH and luteinizing **hormone** (LH) from about 5 years before the menopause. Within the 6 month period around the menopause there was a further increase which culminated within the first **postmenopausal** year for LH and 2-3 years **postmenopause** for FSH. Thereafter, a continuous decrease in LH occurred over the following 8 years. With respect to FSH, there was a slight decline starting about 4 years **postmenopause** . During the **premenopausal** period an increasing frequency of inadequate luteal function or anovulation occurred and, in the **postmenopausal** years, the serum levels of progesterone (P) were invariably low. Gradually, the ratio between estrone (E1) and 17-beta-estradiol (E2) increased, reflecting the declining follicular steroidogenesis. A marked decrease in estrogen levels occurred during the 6 month period around the menopause, most pronounced in E2. During the next 3 years, the levels of E2 and E1 showed an essentially parallel, moderate decline. Around the menopause, serum levels of **testosterone** (T), delta 4-androstenedione (A) and sex **hormone** -binding globulin (SHBG) showed small but significant decreases. From about 3 years **postmenopause** , the levels were relatively constant over the following 5 years. A decrease in BMD was observed in the **postmenopause** , and from about 3 years **postmenopause** , estradiol correlated positively with BMD. Before, as well as after the menopause, body mass index (BMI) showed an inverse correlation with SHBG. **Postmenopausal** androstenedione correlated positively with E1, E2 and T. BMI correlated positively with E1 and E2. The concentrations of the free fraction of E2 and T are dependent on the levels of SHBG, which in turn has a negative correlation with BMI. The impact of this will influence the severity of symptoms, the degree of bone loss and the need for supplementary therapy.
Tags: Female; Human
Descriptors: Bone Density; *Climacteric--metabolism--ME; *Gonadal Steroid Hormones--blood--BL; * **Gonadotropins** , Pituitary--blood--BL; *Sex **Hormone** -Binding Globulin--analysis--AN; Androstenedione--blood--BL; Body Mass Index; Estradiol--blood--BL; Estrone--blood--BL; Follicle Stimulating **Hormone** --blood--BL; Longitudinal Studies; Luteinizing **Hormone** --blood --BL; Menopause--metabolism--ME; Middle Aged; **Postmenopause** --metabolism --ME; **Premenopause** --metabolism--ME; Prospective Studies; **Testosterone** --blood--BL
CAS Registry No.: 0 (Gonadal Steroid Hormones); 0 (Gonadotropins, Pituitary); 0 (Sex Hormone-Binding Globulin); 50-28-2 (Estradiol); 53-16-7 (Estrone); 58-22-0 (Testosterone); 63-05-8 (Androstenedione); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)
Record Date Created: 19950620
Record Date Completed: 19950620

7/9/41
DIALOG(R)File 155:MEDLINE(R)

11734090 PMID: 11910598
Weight gain and the menopause: a 5-year prospective study.
Guthrie J R; Dennerstein L; Dudley E C
Office for Gender and Health, University of Melbourne, Charles Connibere Building, RMH, Vic. 3050, Australia.
Climacteric - the journal of the International Menopause Society (United States) Sep 1999, 2 (3) p205-11, ISSN 1369-7137 Journal Code: 9810959
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
OBJECTIVE: To investigate prospectively changes in weight, skin-fold **measurements** , waist circumference and waist/hip ratio in relation to changes in menopausal status, **hormone** therapy use and life-style factors. METHOD: The study was a 5-year follow-up of volunteers from a population-based cohort of Australian-born women aged 46-57 years at baseline: 106 **premenopausal** , 106 **perimenopausal** and 21 **hormone** therapy users. RESULTS: Mean (SD) weight gain of the entire cohort over 5 years was 2.1 (5.1) kg. Baseline age was negatively associated with weight change (regression coefficient = -0.4, SE 0.1, $p < 0.05$). After 5 years, 20 women remained **premenopausal** , 80 were **perimenopausal** , 112 had become naturally **postmenopausal** and 21 remained on **hormone** therapy. Changes in weight were greater than zero ($p < 0.05$) in all groups except for the women who remained on **hormone** therapy. There was no significant difference in weight gain between women who remained **premenopausal** and those who had a natural menopause. Increases in suprailiac skin-fold **measurements** ($p < 0.05$) and in waist circumference and waist/hip ratio occurred in women who experienced the menopausal transition but not in those who took **hormone** therapy continuously. There was no association between weight change and baseline weight, exercise, alcohol intake or smoking. CONCLUSION: Weight gain was not related to change in menopausal status nor to any life-style factors **measured** . Women who were older at baseline gained less weight than the younger members. Suprailiac skin-fold **measurements** , waist circumference and waist/hip ratio all increased during the menopausal transition. Continuous **hormone** therapy users showed no gain in mean weight, suprailiac skin-fold **measurements** or waist **measurements** over the follow-up period.
Tags: Female; Human; Support, Non-U.S. Gov't
Descriptors: *Menopause; *Weight Gain; Australia; Body Constitution; Cohort Studies; Estrogen Replacement Therapy; Life Style; Middle Aged; **Postmenopause** ; **Premenopause** ; Prospective Studies; Regression Analysis; Skinfold Thickness
Record Date Created: 20020325
Record Date Completed: 20020419

7/9/54
DIALOG(R)File 155:MEDLINE(R)

09717673 PMID: 1303915
Levels of thyroid hormones and thyrotropic hormone **in serum of women with** perimenopausal **arterial hypertension]**
Stezenie hormonow tarczycy i hormonu tyreotropowego w surowicy u kobiet z nadcisnieniem tetniczym w okresie przekwitania.
Stanosz S
Kliniki Ginekologii Instytutu Ginekologii i Poloznictwa AM, Szczecinie.
Ginekologia polska (POLAND) 1992, 63 (3) p130-3, ISSN 0017-0011 Journal Code: 0374641
Document type: Journal Article ; English Abstract
Languages: POLISH

Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Test carried out in 96 women aged between 43 to 55 years (50.46 +/- 4.7), who did not take any drugs during the last 3 months. The women were divided into two groups: **premenopausal** and early **postmenopausal** . Each group was subdivided according to blood pressure: with normal pressure and with arterial hypertension. The concentration of T4, T3 and TSH were **measured** using a radioimmunologic method. The saturation of carrier proteins was established with the T3/ **test** , the result of which was used to divide T4 and T3 and to obtain FT4I and FT3I respectively. It was found that women with arterial hypertension have significantly higher ($p < 0.001$) TSH concentration. The concentration T3 and FT3I were significantly higher ($p < 0.01$) in women with arterial hypertension in the **postmenopausal** period.

Tags: Female; Human
Descriptors: *Hypertension--blood--BL; *Menopause--blood--BL; *Thyroid Hormones--blood--BL; *Thyrotropin--blood--BL; Adult; Middle Aged
CAS Registry No.: 0 (Thyroid Hormones); 9002-71-5 (Thyrotropin)
Record Date Created: 19930701
Record Date Completed: 19930701

7/9/58
DIALOG(R)File 155:MEDLINE(R)

08096813 PMID: 2495752

Menopausal status and cut off levels of steroid receptor ligand binding assays **in breast cancer.**

Norgren A; Forsberg A H; Lindgren A; Sallstrom J F
Department of Pathology, University Hospital, Uppsala, Sweden.

Anticancer research (GREECE) Jan-Feb 1989, 9 (1) p173-6, ISSN 0250-7005 Journal Code: 8102988
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

494 tubuloductal breast carcinomas obtained at operation were **assayed** for ER and PgR with short-term ligand incubation and isoelectric focusing. Plasma FSH and E2 concentrations available from 156 of the patients showed strictly **premenopausal** endocrine conditions in patients 45 years or younger; strictly **postmenopausal** conditions were found at 55 years or older. ER concentrations were significantly lower in biopsies from **premenopausal** compared with those from **postmenopausal** patients. ER concentrations **assayed** in intervening **perimenopausal** age period were not statistically different from the **premenopausal** period. An arbitrarily chosen cut off level to **differentiate** receptor low from receptor high tumours divided **premenopausal assays** into two equal parts; those from **postmenopausal** patients were cut at the second lower percentile. Arbitrary cut off levels ignorant of menopausal status should be replaced by fractionation of low and high receptor tumours on a percentile or quartile basis. Clinically, subgroups or subpopulations of patients should be identified with regard to endocrine and/or receptor status and evaluated separately.

Tags: Female; Human
Descriptors: *Breast Neoplasms--analysis--AN; *Menopause; *Receptors, Estrogen--analysis--AN; *Receptors, Progesterone--analysis--AN; Adult; Age Factors; Aged; Estradiol--blood--BL; Follicle Stimulating **Hormone** --blood --BL; Middle Aged
CAS Registry No.: 0 (Receptors, Estrogen); 0 (Receptors, Progesterone) ; 50-28-2 (Estradiol); 9002-68-0 (Follicle Stimulating Hormone)
Record Date Created: 19890523
Record Date Completed: 19890523

7/9/63
DIALOG(R)File 155:MEDLINE(R)

04708074 PMID: 623728

Assessment of ovarian function in perimenopausal **women after stopping oral contraceptives.**

Donald R A; Baker D A; Metcalf M G; Turner E D

British journal of obstetrics and gynaecology (ENGLAND) Jan 1978, 85 (1) p70-3, ISSN 0306-5456 Journal Code: 7503752
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: AIM; INDEX MEDICUS

Ovarian function was assessed by **measuring** plasma gonadotrophins, urinary oestrogen and urinary pregnanediol levels, every week for four weeks, in 34 women, aged 45 to 57 years, after withdrawal of oral contraceptive therapy. A rise in urinary pregnanediol excretion occurred in 14 patients suggesting that ovulation had occurred and that these patients were " **premenopausal** ". Elevated serum gonadotrophin values and persistently low urinary oestrogen and pregnanediol excretion occurred in 13 patients suggesting ovarian failure or a " **postmenopausal** " state. However, one apparently " **postmenopausal** " patient later showed hormonal evidence of ovulation, indicating fluctuation in ovarian function. There were 7 patients with "indeterminate" **hormone** profiles whose ovarian function was difficult to assess. All patients excreting more than 45 nmol of oestrogen a day had a spontaneous period within 45 days of stopping **hormone** therapy.

Tags: Female; Human
Descriptors: *Contraceptives, Oral; *Menopause; *Ovulation; Adult; Estrogens--urine--UR; Follicle Stimulating **Hormone** --blood--BL; Luteinizing **Hormone** --blood--BL; Middle Aged; Ovulation Detection; Pregnanediol--urine--UR; Time Factors
CAS Registry No.: 0 (Contraceptives, Oral); 0 (Estrogens); 26445-07-8 (Pregnanediol); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)
Record Date Created: 19780417
Record Date Completed: 19780417

?logoff hold
24aug04 16:57:49 User228206 Session D2221.7
$0.98 0.306 DialUnits File155
$4.41 21 Type(s) in Format 9
$4.41 21 Types
$5.39 Estimated cost File155
$0.24 TELNET
$5.63 Estimated cost this search
$5.63 Estimated total session cost 0.306 DialUnits

Status: Signed Off. (1 minutes)

, **perimenopausal** , **postmenopausal** without ovarian **hormone** replacement therapy (HRT), and **postmenopausal** with HRT. Information on lifestyle factors was obtained with interviews and questionnaires. Bone mineral density at the calcaneus was assessed with single-photon absorptiometry, and several serum and urine markers of bone metabolism were **measured** . **Postmenopausal** women without HRT had significantly higher levels of fasting serum alkaline phosphatase, osteocalcin, total and ionized calcium, phosphate, and fasting urinary hydroxyproline than those in the three other study groups. No difference was found in bone mineral density between the **premenopausal** and **postmenopausal** groups. **Postmenopausal** women without HRT showed a marked correlation between serum osteocalcin and urine hydroxyproline. Both markers showed significant correlations with serum calcium, phosphate, and alkaline phosphatase. Multivariate analyses showed a statistically significant association of ovarian **hormone** status and body mass index with most **measured** markers of bone metabolism. The association between alcohol consumption and serum osteocalcin was highly significant. Cigarette smoking was associated with levels of serum alkaline phosphatase and total and ionized calcium. A weak association was found between coffee drinking and serum alkaline phosphatase.

Tags: Female; Human

Descriptors: Bone Density--physiology--PH; *Bone and Bones--metabolism --ME; *Life Style; *Menopause; * **Premenopause** ; Absorptiometry, Photon; Alcohol Drinking; Biological Markers--blood--BL; Calcaneus; Coffee; Cohort Studies; Estrogen Replacement Therapy; Finland; Middle Aged; Multivariate Analysis; **Postmenopause** ; Smoking

CAS Registry No.: 0 (Biological Markers)

Record Date Created: 19940922

Record Date Completed: 19940922

7/9/12

DIALOG(R)File 155:MEDLINE(R)

14145612 PMID: 9844847

Menopausal status: subjectively and objectively defined.

Garamszegi C; Dennerstein L; Dudley E; Guthrie J R; Ryan M; Burger H

Department of Psychiatry, University of Melbourne, Carlton, Victoria, Australia.

Journal of psychosomatic obstetrics and gynaecology (ENGLAND) Sep 1998, 19 (3) p165-73, ISSN 0167-482X Journal Code: 8308648

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

This study aims to assess the relationship between self-rated and menstrually defined menopausal status, assesses criteria women use in perceiving their own menopausal status and compares symptom reporting and hormonal levels for self-rated and menstrually defined menopausal status. Women in the third year of the longitudinal phase of the Melbourne Women's Midlife Health Project (n = 332) were asked to assess their own menopausal status and the basis for this assessment. They were also specifically questioned on current menstrual cycle characteristics and levels of follicle-stimulating **hormone** (FSH), estradiol and inhibin were **measured** . For 67% of the women, the two definitions of menopausal status were in agreement. In women menstrually defined as **premenopausal** , self-rated menopausal status of peri- or **postmenopausal** appeared to be based on the occurrence of symptoms. In women menstrually defined as **postmenopausal** , persistence of hot flushes was taken to mean that 'the menopause was still in progress' despite absence of menses for more than 12 months. In women menstrually defined as **perimenopausal** yet who self-rated as **premenopausal** , FSH was lower ($p < 0.01$) and inhibin higher ($p = 0.05$) than women who self-rated as peri- or **postmenopausal** . Women's perceptions of the menopause are based on symptoms. Self-rated menopausal status appears to relate more closely to a women's endocrine status than definitions based on purely menstrual cycle characteristics.

01338400

Test methods and devices for analyte isoforms
Testverfahren und Vorrichtungen fur Analytisoformen
Methodes d'essai et dispositifs pour les isoformes d'un analyte

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200141	592
SPEC A	(English)	200141	3446
Total word count - document A			4038
Total word count - document B			0
Total word count - documents A + B			4038

...ABSTRACT between states of an analyte that can exist in different forms, such as follicle stimulating **hormone** (**FSH**). The method or test device uses two contemporaneous assays, the first of which does not...

...second of which does, and the assay results are compared. A novel pair of anti- **FSH** monoclonal antibodies that can be used together in a sandwich-format assay to differentiate pre-menopausal and post-menopausal **FSH** samples is disclosed.

01338399

Test methods and devices for analyte isoforms
Testverfahren und Vorrichtungen fur Analytisoformen
Methodes d'essai et dispositifs pour les isoformes d'un analyte

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200141	809
SPEC A	(English)	200141	3725
Total word count - document A			4534
Total word count - document B			0
Total word count - documents A + B			4534

...ABSTRACT between states of an analyte that can exist in different forms, such as follicle stimulating **hormone** (**FSH**). The method or test device uses a pair of specific binding agents, especially monoclonal antibodies ...

```
File 347:JAPIO Nov 1976-2004/Apr(Updated 040802)
         (c) 2004 JPO & JAPIO
*File 347: JAPIO data problems with year 2000 records are now fixed.
Alerts have been run.  See HELP NEWS 347 for details.
  File 144:Pascal 1973-2004/Aug W3
         (c) 2004 INIST/CNRS

      Set  Items  Description
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Cost is in DialUnits
?ds

Set     Items   Description
S1        170   DEVICE? (10N) MENOPAUS?
S2        149   RD (unique items)
S3         74   S2 AND (HORMON? OR FSH? OR HCG? OR ESTROG? OR GONADOTROP?)
S4         31   S3/2000:2004
S5         43   S3 NOT S4
S6         43   TARGET - S5
?t s4/3,kwic/15 17 27 28
```

0928408

METHOD AND SYSTEM FOR DIAGNOSING ANDROPAUSE IN MALES

PROCEDE ET SYSTEME PERMETTANT DE DIAGNOSTIQUER L'ANDROPAUSE CHEZ LES HOMMES

Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 6242
Publication Year: **2002**

Patent and Priority Information (Country, Number, Date):
Patent: ... **20020808**
Fulltext Availability:
Detailed Description
Claims
Publication Year: **2002**

Detailed Description
... the diagnosis of androgen decline in males now commonly referred to as andropause or male **menopause** , In particular, the present invention relates to a diagnostic method, **device** and test kit for indicadilg andropausal status and/or reduced gonadal function iii males.

Background...

...40, and the incidence increases with each decade of life. Associated with declines in androgenic **hormone** production are declines in male gonadal function which tend to develop very gradually over many...

...which a decline in ovarian function results in a corresponding decline in the levels of **estrogen** .

In both andropause and female menopause the decline in biologically active levels of the sex **hormones** causes the clinical signs and symptoms associated with these two conditions. The declining levels of gonadal **hormones** are a direct result of a progressive reduction of gonadal function leading to a decrease in the production and secretion of the sex **hormones** . In men declining testicular function associated with andropause lowers the levels of androgens (i,e...

0005722524 **IMAGE Available
Derwent Accession: 2002-619284
US 20040137520
Method and system for diagnosing andropause in males

Fulltext Word Count: 8057
Number of Claims: 45
Exemplary or Independent Claim Number(s): 1,7,12,19,30,35,40
Number of Drawing Sheets: 1
Number of Figures: 2

... 20040715

Summary of the Invention:
...the diagnosis of androgen decline in males now commonly referred to as andropause or male **menopause** . In particular, the present invention relates to a diagnostic method, **device** and test kit for indicating andropausal status and/or reduced gonadal function in males...

...to as male menopause, is defined as a progressive decline in the production of androgenic **hormones** which commonly occurs in ageing adult males. The age of onset of andropause is very...40, and the incidence increases with each decade of life. Associated with declines in androgenic **hormone** production are declines in male gonadal function which tend to develop very gradually over many...

...which a decline in ovarian function results in a corresponding decline in the levels of **estrogen** .

01338400

Test methods and devices for analyte isoforms
Testverfahren und Vorrichtungen fur Analytisoformen
Methodes d'essai et dispositifs pour les isoformes d'un analyte

PATENT ASSIGNEE:
UNILEVER PLC, (200923), Unilever House, Blackfriars, London EC4P 4BQ, (GB), (Applicant designated States: all)
UNILEVER N.V., (200916), Weena 455, 3013 AL Rotterdam, (NL), (Applicant designated States: all)
INVENTOR:
Butlin, Lorraine Diane, Unilever Research Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
Coley, John, Unilever Research Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
Eida, Stephen James, Unilever Research Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
Gani, Mohamed Mutwahar, Unilever Research Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
LEGAL REPRESENTATIVE:
Lipscombe, Martin John et al (80581), Keith W Nash & Co, Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP, (GB)
PATENT (CC, No, Kind, Date): EP 1143250 A2 011010 (Basic)
APPLICATION (CC, No, Date): EP 2001303132 010402;
PRIORITY (CC, No, Date): EP 2000302810 000403
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE; TR
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: G01N-033/543; G01N-033/76; G01N-033/577; C07K-016/26
ABSTRACT WORD COUNT: 83

01338399

Test methods and devices for analyte isoforms
Testverfahren und Vorrichtungen fur Analytisoformen
Methodes d'essai et dispositifs pour les isoformes d'un analyte

PATENT ASSIGNEE:
UNILEVER PLC, (200923), Unilever House, Blackfriars, London EC4P 4BQ, (GB), (Applicant designated States: all)
UNILEVER N.V., (200916), Weena 455, 3013 AL Rotterdam, (NL), (Applicant designated States: all)
INVENTOR:
Butlin, Lorraine Diane, c/o Unilever Rch. Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
Coley, John, c/o Unilever Rch. Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
Eida, Stephen J., Unilever Rch. Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
Gani, Mohamed M., c/o Unilever Rch. Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, (GB)
LEGAL REPRESENTATIVE:
Lipscombe, Martin John et al (80581), Keith W Nash & Co, Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP, (GB)

PATENT (CC, No, Kind, Date): EP 1143249 A2 011010 (Basic)
APPLICATION (CC, No, Date): EP 2001303130 010402;
PRIORITY (CC, No, Date): EP 2000302811 000403
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE; TR
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: G01N-033/543; G01N-033/76; G01N-033/577; C07K-016/26
ABSTRACT WORD COUNT: 96

concentration of activin-AB were between 1.3% and 2.67%. The between-plate coefficient of variation was 5.5%. Cross-reactivity experiments showed the high specificity of the assay for activin-AB, with inhibin-A, inhibin-B, follistatin, activin-A and activin-B all cross-reacting < 0.2%. Incubation with high concentrations of follistatin (500 ng/ml) prior to assay did not affect the recovery of activin-AB. Samples of bovine, porcine, ovine and human FF gave dose responses parallel to that of the standard, as did bovine granulosa cell-conditioned media. In human and porcine FF, levels of activin-A and activin-AB were similar whereas, in bovine and ovine FF, activin-A levels were approximately threefold higher than activin-A, nearly all of the endogenous activin-AB in bovine FF was detected in the eluate from gel permeation chromatography with an M(r) of > 700000 indicating its association with higher molecular weight binding protein(s). By contrast, after denaturation, immunoreactive activin-AB was detected with an M(r) of approximately 25000 consistent with the complete dissociation from binding proteins. Activin-A was detected in relatively high concentrations in human FF (approximately 5 ng/ml), homogenized placental extracts (4.35-95.5 ng/g), sera from pregnant women (> 4 ng/ml) and amniotic fluid (3-13 ng/ml), and in much lower concentrations in **postmenopausal** serum (500 pg/ml), normal cycle serum (100-200 pg/ml), serum from gonadotrophin-treated women (200 pg/ml), and normal adult male serum (225 pg/ml). Activin-A was also found in the culture media from explants of human amnion, chorion, maternal decidua and placenta. In marked contrast, activin-AB was undetectable (< 0.19 ng/ml) in all of these samples with the exception of human FF (approximately 7 ng/ml). In conclusion, we have developed a sensitive and specific ELISA to measure total (bound+free) activin-AB. Preliminary results show a more restricted distribution of this **isoform** compared with activin-A. The presence of high levels of both activin-A and activin-AB in FF suggests a function for both **isoforms** in the developing ovarian follicle.

Tags: Female; Human; Pregnancy; Support, Non-U.S. Gov't

Descriptors: *Follicular Fluid--chemistry--CH; *Oligopeptides; Activins; Amnion--chemistry--CH; Animals; Cattle; Chorion--chemistry--CH; Decidua --chemistry--CH; Enzyme-Linked Immunosorbent Assay; Inhibins--analysis--AN; Isomerism; Peptides--analysis--AN; Placenta--chemistry--CH; **Postmenopause** --blood--BL; Reproducibility of Results; Sheep; Swine

CAS Registry No.: 0 (Oligopeptides); 0 (Peptides); 0 (activin B); 104625-48-1 (Activins); 137833-32-0 (myelopeptides); 57285-09-3 (Inhibins)

Record Date Created: 19970612

Record Date Completed: 19970612

8/9/44

13223241 PMID: 8891527

Interest of epitopic dissection in immunoanalysis of proteins and peptides: review of theoretical and practical aspects.

Niccoli P; Ferrand V; Lejeune P J; Carayon P

Laboratoire de Biochimie Endocrinienne et Metabolique, Institut National de la Sante et de la Recherche Medicale, Faculte de Medecine, Marseille, France.

European journal of clinical chemistry and clinical biochemistry - journal of the Forum of European Clinical Chemistry Societies (GERMANY) Sep 1996, 34 (9) p741-8, ISSN 0939-4974 Journal Code: 9105775

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The literature abounds with reports showing discrepancies in immunoassays of proteins and peptides. Whereas the isomorphism and polymorphism of proteins remains largely hidden in immunoassays making use of polyclonal antibodies, the use of monoclonal antibodies uncovered the difficulty of accurately assaying microheterogeneous analytes. Indeed, most proteic

hormones are not entities with unique structures but rather mixtures of molecular forms with slight differences in structure which may reflect large variations in biological and immunological activities; the monoclonal antibodies appeared clearly less suited than the polyclonal for testing a mixture of **isoforms** . Protein microheterogeneity also has an impact on assay standardisation, since reference preparations may contain several **isoforms** of the analyte. Using recombinant glycoprotein does not solve the problem. Regarding the problem of discrepancy in immunoanalysis of proteins and peptides, we could establish, in a previous work, that discrepancy among lutropin assay kits may be related to various causes: i) differences in standard preparation and calibration curves; ii) microheterogeneity of lutropin molecules leading to missing some **isoforms** due to the restricted epitopic specificity of the monoclonal antibodies used in the kits. The epitopic dissection we engaged in appeared thus instrumental in explaining these discrepancies. It allowed us to enumerate epitopes on the surface of lutropin molecules, to elucidate the immunological structure and, finally, to characterize monoclonal antibodies used in commercially available lutropin assay kits with regard to their epitopic specificity. This work allowed us to interpret the discrepancy in serum lutropin concentration which was related to the use of monoclonal antibody with given specificity. Epitopic dissection may thus be instrumental in explaining discrepancy among immunoassays of proteins and peptides and in improving the accuracy of kits. (19 Refs.)

Tags: Female; Human; Male; Support, Non-U.S. Gov't

Descriptors: *Epitopes--chemistry--CH; *Immunoassay--methods--MT; *Peptides--chemistry--CH; *Proteins--chemistry--CH; Antibodies, Monoclonal; Kidney Failure--blood--BL; Luteinizing Hormone--blood--BL; **Menopause** --blood--BL; Polycystic Ovary Syndrome--blood--BL; Polymorphism (Genetics); Reagent Kits, Diagnostic--standards--ST; Reference Values

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Epitopes); 0 (Peptides); 0 (Proteins); 0 (Reagent Kits, Diagnostic); 9002-67-9 (Luteinizing Hormone)

Record Date Created: 19970206

Record Date Completed: 19970206

8/9/47

DIALOG(R)File 155:MEDLINE(R)

13119601 PMID: 8787959

Undetectable luteinizing hormone levels using a monoclonal immunometric assay.

Barbe F; Legagneur H; Watrin V; Klein M; Badonnel Y

Service de Biologie Medicale, Maternite Regionale, Nancy, France.

Journal of endocrinological investigation (ITALY) Nov 1995, 18 (10) p806-8, ISSN 0391-4097 Journal Code: 7806594

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Previous studies have shown wide discrepancies among the results obtained with different immunometric assays. We present five cases (out of 4000 women) whose plasma luteinizing hormone was not detected using a LH immunometric assay (LH Stratus Baxter) but was recognized by other kits. These cases concerned one 28-year-old woman presenting with infertility and four **postmenopausal** women. The LH Amerlite kit gave detectable but low results. The results obtained with the other kits were > 7 IU/l. FSH levels were > 7 IU/l. In one case, sera were taken before and after the menopause; differences between the LH results increased. Discrepancies among LH assay kits have been attributed to variation both in standard curve calibration and in epitope specificity of the kit monoclonal antibodies. The Baxter kit might misrecognize some **isoforms** present in **postmenopausal** women. The present data illustrate the potential false results with such immunoassays in routine clinical laboratory testing. When undetectable LH results are not clinically explained or when disparities between LH and FSH are

observed, we suggest using a second methodology or a bioassay if necessary. Improvement in LH assays and standardization might resolve the problem of discrepancies between the LH results.

Tags: Comparative Study; Female; Human

Descriptors: *Antibodies, Monoclonal; *Immunoassay--methods--MT; *Luteinizing Hormone--blood--BL; Adult; False Negative Reactions; Follicle Stimulating Hormone--blood--BL; Immunoassay--statistics and numerical data --SN; Middle Aged; **Postmenopause** ; Reagent Kits, Diagnostic--statistics and numerical data--SN

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Reagent Kits, Diagnostic); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19961021

Record Date Completed: 19961021

8/9/56

DIALOG(R)File 155:MEDLINE(R)

12549532 PMID: 7889633

Effect of chronic daily oral administration of 17 beta-oestradiol and norethisterone on the isoforms **of serum gonadotrophins in post-menopausal women.**

Wide L; Naessen T; Phillips D J

Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.

Clinical endocrinology (ENGLAND) Jan 1995, 42 (1) p59-64, ISSN 0300-0664 Journal Code: 0346653

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

OBJECTIVE: Chronic treatment with 17 beta-oestradiol (E2) implants has been found to counteract the formation of more acidic **isoforms** of the gonadotrophins in post-menopausal women. Oral medication with an oestrogen in combination with a progestagen is a common hormone replacement therapy (HRT) in post-menopausal women. The present study investigated the effect of such a therapy on the concentration and charge of the gonadotrophin **isoforms** in serum. DESIGN: Serum samples were obtained from 20 post-menopausal women, mean age 60 years (range 50-72 years), treated with continuous daily oral medication of 2 mg E2 combined with 1 mg norethisterone acetate (NETA). FSH, LH and E2 in the serum was measured with fluoroimmunoassays. The median charge and charge heterogeneity of the FSH and LH **isoforms** were determined for each serum by electrophoresis in 0.1% agarose suspension. Sera from 20 post-menopausal women without a history of HRT served as controls. The results were compared with those from previous studies on post-menopausal women treated with E2 implants and on women with normal menstrual cycles. RESULTS: The E2 level in the oral-E2 + NETA treated women was 198-610 pmol/l, within the range expected during the mid-luteal phase of the normal menstrual cycle and similar to that of the group of women with an E2 implant. The mean LH level was similar to that of the luteal phase of the cycle and significantly lower than that of the controls ($P < 0.001$), the E2 implant group ($P < 0.001$) and at the follicular phase of the cycle ($P < 0.01$). The mean FSH level was similar to that of the follicular phase and the E2 implant group but lower than that of the controls ($P < 0.001$) and higher than at the luteal phase of the cycle ($P < 0.01$). The mean values for median charge of both FSH and LH were less acidic than those of the controls ($P < 0.001$) but more acidic than those for the E2 implant group ($P < 0.01$; $P < 0.001$) and for different phases of the menstrual cycle ($P < 0.05$; $P < 0.001$). The mean degree of charge heterogeneity of FSH was larger ($P < 0.01$), while that of LH was smaller ($P < 0.01$), than for the controls. The mean concentrations of SHBG in the oral E2 + NETA group, the E2 implant group and the controls were similar. CONCLUSION: Chronic oral therapy with 2 mg 17 beta-oestradiol combined with 1 mg norethisterone in post-menopausal women efficiently decreased the serum gonadotrophin levels but only partly counteracted the

formation of the more acidic **isoforms** of FSH and LH after menopause. The differences in the charge for both FSH and LH between the E2 implant and the oral E2 + NETA treated groups may be due to the differences in route of administration of E2 or to the effect of norethisterone or both.

Tags: Female; Human

Descriptors: Estradiol--administration and dosage--AD; *Estrogen Replacement Therapy; *Follicle Stimulating Hormone --antagonists and inhibitors--AI; *Luteinizing Hormone--antagonists and inhibitors--AI; *Norethindrone--administration and dosage--AD; * **Postmenopause** --blood--BL ; Aged; Drug Therapy, Combination; Estradiol--blood--BL; Menstrual Cycle --blood--BL; Middle Aged; Sex Hormone-Binding Globulin--metabolism--ME

CAS Registry No.: 0 (Sex Hormone-Binding Globulin); 50-28-2 (Estradiol); 68-22-4 (Norethindrone); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19950420

Record Date Completed: 19950420

8/9/93

11666798 PMID: 11836327

Characterization of inhibin forms and their measurement by an inhibin alpha-subunit ELISA in serum from postmenopausal **women with ovarian cancer.**

Robertson D M; Stephenson T; Pruysers E; McCloud P; Tsigos A; Groome N; Mamers P; Burger H G

Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia. david.robertson@med.monash.edu.au

Journal of clinical endocrinology and metabolism (United States) Feb 2002, 87 (2) p816-24, ISSN 0021-972X Journal Code: 0375362

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

The aim of this study was to characterize the molecular wt forms of inhibins A and B and its free alpha-subunit present in serum from women with ovarian cancer as a basis for developing improved monoclonal antibody-based inhibin assays for monitoring ovarian cancer. Three new inhibin alpha-subunit (alphaC) ELISAs were developed using monoclonal antibodies directed to three nonoverlapping peptide regions of the alphaC region of the inhibin alpha-subunit. To characterize serum inhibin molecular wt forms present in women with ovarian cancer, existing inhibin immunoassays (inhibin A, inhibin B, and pro-alphaC) and the new alphaC ELISAs were applied to sera from women with granulosa cell tumors and mucinous carcinomas previously fractionated using a combined immunoaffinity chromatography, preparative SDS-PAGE, and electroelution procedure. The distribution and molecular size of dimeric inhibins and alpha-subunit detected were consistent with known mol wt forms of inhibins A and B and inhibin alpha-subunit and their precursor forms present in serum and follicular fluid from healthy women. The alphaC ELISAs recognized all known forms of inhibin and the free inhibin alpha-subunit, although differences between alphaC ELISAs were observed in their ability to detect high mol wt forms. To assess which of the alphaC ELISAs was preferred in application to ovarian cancer, the alphaC ELISAs were applied to serum from a range of normal **postmenopausal** women (n = 61) and **postmenopausal** women (n = 152) with ovarian (serous, mucinous, endometrioid, clear cell carcinomas, and granulosa cell tumors) and nonovarian (breast and colon) cancers. Despite differences in their ability to detect high mol wt forms of inhibin, the alphaC ELISAs showed similar sensitivity (i.e. proportion of cancer patients correctly detected) and specificity (proportion of controls correctly detected) indexes in the detection of mucinous carcinomas (84% and 95%) and granulosa cell tumors (100% and 95%) compared with earlier inhibin RIA or polyclonal antibody-based immunofluorometric assays. A combination of the alphaC ELISAs with the CA125 assay, an ovarian tumor

marker that has a high sensitivity and specificity for other ovarian cancers (serous, clear cell, and endometrioid), resulted in an increase in sensitivity/specificity indexes (95% and 95%) for the all ovarian cancer group. These new monoclonal antibody-based inhibin alphaC ELISAs now provide practical and sensitive assays suitable for evaluation as diagnostic tests for monitoring ovarian cancers.

Tags: Female; Human; Support, Non-U.S. Gov't

Descriptors: Enzyme-Linked Immunosorbent Assay--methods--MT; *Inhibins --blood--BL; *Ovarian Neoplasms--blood--BL; * **Postmenopause** --blood--BL; Adenocarcinoma, Mucinous--blood--BL; Aged; CA-125 Antigen--blood--BL; Cystadenocarcinoma, Serous--blood--BL; Granulosa Cell Tumor--blood--BL; Immunoassay; Middle Aged; Protein **Isoforms** --blood--BL; ROC Curve; Sensitivity and Specificity

CAS Registry No.: 0 (CA-125 Antigen); 0 (Protein Isoforms); 0 (inhibin-alpha subunit); 57285-09-3 (Inhibins)

Record Date Created: 20020211

Record Date Completed: 20020301

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$3.78 18 Type(s) in Format 9

$3.78 18 Types

$4.72 Estimated cost File155

$0.24 TELNET

$4.96 Estimated cost this search

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Status: Signed Off. (1 minutes)

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Trying 31060000009998...Open

DIALOG INFORMATION SERVICES
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File 155:MEDLINE(R) 1951-2004/Aug W4
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      Set  Items  Description
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Set     Items   Description
S1         40   'MENOPAUSE --IMMUNOLOGY --IM' OR 'MENOPAUSE--DETERMINANTS'
S2        674   'MENOPAUSE --BLOOD --BL'
S3         16   'MENOPAUSE, PREMATURE --IMMUNOLOGY --IM'
S4         16   'PREMENOPAUSE --IMMUNOLOGY --IM'
S5         38   'PREMENOPAUSE --URINE --UR'
S6       1659   PERIMENOPAU?
S7      25828   POSTMENOPAU?
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 1/9/40
DIALOG(R)File 155:MEDLINE(R)
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06015977   PMID:   7174725   Record   Identifier:   S-3220-39-6;   9003881;
00123372
  Early menopause and its determinants.
  Mahadevan K; Murthy M S; Reddy P R; Bhaskaran S
  Journal of biosocial science (ENGLAND)   Oct 1982,  14  (4)  p473-9,
ISSN 0021-9320   Journal Code: 0177346
  TJ: JOURNAL OF BIOSOCIAL SCIENCE
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Other Citation Owner: CPC; IND; POP
  Record type: Completed
  Subfile:   INDEX MEDICUS
  Tags: Female; Human
  Descriptors:  *Menopause;  *Menopause,  Premature;  Adult;  Ascorbic Acid
--blood--BL; Blood Proteins--analysis--AN; Hemoglobins--analysis--AN; India
; Middle Aged; Nutrition; Sexual Behavior; Vaginal Smears
```

09676635 PMID: 8473374

Concanavalin A affinity chromatography of human serum gonadotropins: evidence for changes of carbohydrate structure in different clinical conditions.

Papandreou M J; Asteria C; Pettersson K; Ronin C; Beck-Peccoz P

Istituto di Scienze Endocrine, Universita di Milano, Ospedale Maggiore IRCCS, Italy.

Journal of clinical endocrinology and metabolism (UNITED STATES) Apr 1993, 76 (4) p1008-13, ISSN 0021-972X Journal Code: 0375362

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

We have studied the carbohydrate of circulating human gonadotropins (FSH and LH) in different clinical conditions using Concanavalin A (Con A) affinity chromatography. This technique permits separation of molecules differing in the extent of carbohydrate branching. The proportion of molecules that does not bind to Con A was greater in circulating FSH than in LH, reflecting a higher content of multiantennary and/or bisected biantennary complex carbohydrate structures in serum FSH. No significant difference in gonadotropin binding pattern to Con A was found between normal controls and patients with chronic uremia or gonadotropin-secreting pituitary adenomas. On the contrary, sera from **postmenopausal** women and fetuses contained a greater proportion of FSH and LH that bound to Con A, indicating a shift from multiantennary and/or bisecting structures to hybrid and/or high mannose forms, i.e. to the secretion of less mature forms. International Reference Preparations, derived from pituitary extracts, were more retained on Con A than circulating hormones, suggesting that carbohydrate chains of the intrapituitary hormone stock are less mature than those present in the circulation. Less mature forms were also found in FSH, but not in LH, from normal controls after GnRH injection. Finally, a higher proportion of unbound forms, i.e. complex carbohydrate chains, was found in healthy subjects presenting with an immunologically anomalous variant of LH. In conclusion, the current data show that the hormonal status of the individual may differently affect carbohydrate branching of gonadotropins. Alteration in **glycosylation** is likely to be involved in masking at least one epitope specific for intact LH dimer, thus indicating that it may modulate the tertiary structure of glycoprotein hormones.

Tags: Female; Human; Male; Support, Non-U.S. Gov't

Descriptors: *Gonadotropins--blood--BL; Adult; Carbohydrate Conformation; Chromatography, Affinity; Concanavalin A; Follicle Stimulating Hormone --blood--BL; Gonadotropins--chemistry--CH; Gonadotropins--metabolism--ME; Isoelectric Focusing; Luteinizing Hormone--blood--BL; Middle Aged; Pituitary Gland--metabolism--ME; Reference Values

CAS Registry No.: 0 (Gonadotropins); 11028-71-0 (Concanavalin A); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19930518

Record Date Completed: 19930518

8/9/135

09361155 PMID: 1624024

Distribution of follicle-stimulating hormone and luteinizing hormone isoforms **in sera from women with primary ovarian failure compared with that of normal reproductive and** postmenopausal **women.**

Mason M; Fonseca E; Ruiz J E; Moran C; Zarate A

Endocrine Research Unit, Instituto Mexicano del Seguro Social, D.F.

Fertility and sterility (UNITED STATES) Jul 1992, 58 (1) p60-5, ISSN 0015-0282 Journal Code: 0372772

Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

OBJECTIVE: To demonstrate if molecular heterogeneity of gonadotropins correlates with the type of primary gonadal failure. DESIGN AND METHODS: Aliquots of sera from women with hypogonadism were subjected to gel filtration chromatography to be assayed for follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the use of radioimmunoassay. Molecular weight (MW) of **isoforms** was calculated on a calibration curve obtained with molecular markers. The molecular variants were characterized on the basis of elution volume, MW, and partition coefficient. RESULTS: Chromatographic profile of sera from four women with natural menopause exhibited two FSH peaks of immunoreactivity and a heavier LH **isoform** . This pattern was different from that obtained in sera from women of reproductive age who presented a single peak that eluted after the corresponding standard. In six cases of idiopathic premature menopause and three more with gonadotropin-resistant ovary, the chromatographic profile showed a marked and remarkable molecular heterogeneity, particularly LH, and this was more apparent in women with resistant ovary. CONCLUSIONS: Our investigation confirms the relationship between the gonadotropin heterogeneity with the gonadal failure. The duration of the ovarian failure may influence the molecular proportion of gonadotropins and the predominance of heavier MW isohormones.

Tags: Comparative Study; Female; Human; Support, Non-U.S. Gov't
Descriptors: Follicle Stimulating Hormone--blood--BL; *Luteinizing Hormone--blood--BL; * **Menopause** --blood--BL; *Ovarian Failure, Premature --blood--BL; Adult; Chromatography, Gel; Isomerism; Middle Aged; Molecular Weight; Radioimmunoassay
CAS Registry No.: 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)
Record Date Created: 19920811
Record Date Completed: 19920811

8/9/33

14002589 PMID: 9701785

Glycoform **composition of serum gonadotrophins through the normal menstrual cycle and in the post-menopausal state.**

Anobile C J; Talbot J A; McCann S J; Padmanabhan V; Robertson W R
University of Manchester, Department of Medicine, Hope Hospital, Salford, UK.

Molecular human reproduction (ENGLAND) Jul 1998, 4 (7) p631-9, ISSN 1360-9947 Journal Code: 9513710
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The heterogeneity of follicle stimulating hormone (FSH) and luteinizing hormone (LH) was investigated in five women aged 29.4 +/- 3.2 years (mean +/- SD) throughout their menstrual cycles and in five post-menopausal women aged 53.8 +/- 5.6 years. Chromatofocusing (pH range 7-4) revealed menstrual cycle stage- and **postmenopausal** -related differences in the serum gonadotrophin charge. There were differences in the proportion of FSH with an isoelectric point (pI) > 4.3 across phases of the menstrual cycle (P = 0.019): midcycle (MC) 50%; early to mid-follicular (EMF) 36%; late follicular (LF) 37%, luteal (L) 29% and following the menopause (PM) 17%. There was no significant difference in the proportion of LH with pI > 6.55 between midcycle (53%) and EMF, LF or L phases (36, 43 and 32% respectively); although all were greater than that found in the menopause (13%). Concanavalin A chromatography revealed less (P < 0.005) complex FSH and LH **glycoforms** at midcycle (63 and 13%) than in the EMF, LF and L phases (90 and 18; 90 and 20 and 93 and 24% respectively). Menopausal

Menopausal

#33

gonadotrophins were least complex (FSH 34%, LH 4%). There was a direct relationship between serum FSH and FSH pI/complexity, and less acidic FSH was associated with reduced FSH complexity. Increased oestradiol was associated with basic FSH **isoforms** during the menstrual cycle and reduced follicular phase FSH complexity. We conclude that changes in gonadotrophin **glycoforms** occur through the menstrual cycle which are related to changes in the prevailing steroid environment. Following the menopause oestrogenic loss resulted in acidic, relatively simple **glycoforms** .

Tags: Female; Human; Support, Non-U.S. Gov't

Descriptors: Follicle Stimulating Hormone--blood--BL; *Luteinizing Hormone--blood--BL; *Menstrual Cycle; * **Postmenopause** ; Adult; Chromatography, Affinity; Estradiol--blood--BL; **Glycosylation** ; Isoelectric Point; Middle Aged; Ovarian Follicle--physiology--PH; Ovulation ; Progesterone--blood--BL; Reference Values

CAS Registry No.: 50-28-2 (Estradiol); 57-83-0 (Progesterone); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19981028

Record Date Completed: 19981028

8/9/22

DIALOG(R)File 155:MEDLINE(R)

14372750 PMID: 10369198

Inhibins: paracrine and endocrine effects in female reproductive function.

Petraglia F; Zanin E; Faletti A; Reis F M

Department of Surgical Sciences, University of Udine, Italy. Felice.Petraglia@DSC.Uniud.it

Current opinion in obstetrics & gynecology (ENGLAND) Jun 1999, 11 (3) p241-7, ISSN 1040-872X Journal Code: 9007264

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A great deal of new information has arisen in the past 2 years concerning the physiology of inhibins and their clinical relevance in reproductive medicine. It is now recognized that the two inhibin **isoforms** , inhibin A and inhibin B, are produced by the gonads in the course of gamete maturation and have different patterns of secretion during the menstrual cycle. Inhibins are also produced by the placenta and fetal membranes and may be involved in physiological adaptation of pregnancy. Clinically, inhibins may serve as sensitive tumor markers in **postmenopausal** women, or as useful tools for evaluating ovarian reserve in infertile women; they may also be used in the diagnosis of materno-fetal disorders. (53 Refs.)

Tags: Female; Human; Pregnancy

Descriptors: *Endocrine Glands--physiology--PH; *Inhibins--metabolism--ME ; *Paracrine Communication; *Reproduction--physiology--PH

CAS Registry No.: 57285-09-3 (Inhibins)

Record Date Created: 19990802

Record Date Completed: 19990802

8/9/30

DIALOG(R)File 155:MEDLINE(R)

14107347 PMID: 9806574

Low levels of serum inhibin A and inhibin B in women with hypergonadotropic amenorrhea and evidence of high levels of activin A in women with hypothalamic amenorrhea.

Petraglia F; Hartmann B; Luisi S; Florio P; Kirchengast S; Santuz M; Genazzani A D; Genazzani A R

University of Udine, Italy. felicepetraglia@dsc.uniud.it

Fertility and sterility (UNITED STATES) Nov 1998, 70 (5) p907-12, ISSN 0015-0282 Journal Code: 0372772

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

OBJECTIVE: To examine serum levels of inhibin A, inhibin B, and activin A in women with secondary hypergonadotropic or hypothalamic amenorrhea. DESIGN: Retrospective study. SETTING: Universities of Udine, Pisa, and Modena in Italy, and of Wien in Austria. PATIENT(S): Forty women with idiopathic premature ovarian failure (POF), 23 women with hypogonadotropic hypothalamic amenorrhea, 40 healthy **postmenopausal** women, and 40 age-matched women with normal ovarian function (controls). INTERVENTION(S): Blood samples were collected between 8 and 9 AM. MAIN OUTCOME MEASURE(S): Serum levels of inhibin A, inhibin B, and activin A. RESULT(S): Women with POF had lower concentrations of serum inhibin A and inhibin B than women with hypothalamic amenorrhea and fertile controls, and the difference between these concentrations was statistically significant. Levels of inhibin A and inhibin B were low in **postmenopausal** women and were no different than in women with POF. Serum levels of activin A were not significantly different among women with POF, fertile controls, and **postmenopausal** women. Women with hypogonadotropic hypothalamic amenorrhea had higher activin A values than did controls. No significant correlation was found between the level of inhibin A or inhibin B and the length of amenorrhea or the level of FSH. CONCLUSION(S): Low levels of circulating inhibins A and B, but not activin A, reflect ovarian failure in women with POF, whereas women with hypogonadotropic hypothalamic amenorrhea have normal levels of inhibins A and B and high levels of activin A.

Tags: Female; Human

Descriptors: Amenorrhea--blood--BL; *Hypothalamic Diseases--blood--BL; *Inhibins--blood--BL; *Ovarian Failure, Premature--blood--BL; *Protein **Isoforms** --blood--BL; Activins; Adult; Estradiol--blood--BL; Follicle Stimulating Hormone--blood--BL; Luteinizing Hormone--blood--BL; Middle Aged ; Reference Values; Retrospective Studies

CAS Registry No.: 0 (Protein Isoforms); 104625-48-1 (Activins); 50-28-2 (Estradiol); 57285-09-3 (Inhibins); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)

Record Date Created: 19981117

Record Date Completed: 19981117

8/9/37

DIALOG(R)File 155:MEDLINE(R)

13820436 PMID: 9518873

Development, validation and application of an ultra-sensitive two-site enzyme immunoassay for human follistatin.

Evans L W; Muttukrishna S; Groome N P

School of Biological and Molecular Sciences, Oxford Brookes University, Headington, UK.

Journal of endocrinology (ENGLAND) Feb 1998, 156 (2) p275-82, ISSN 0022-0795 Journal Code: 0375363

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Recent studies have found follistatin to be an important regulator of activin bioactivity. Whilst a number of assay formats have been described, all are of limited sensitivity and require the use of isotopes. Many use polyclonal antibodies. Furthermore, a wide range of follistatin preparations have been used as standards, complicating inter-laboratory comparison. We now describe an ultra-sensitive two-site enzyme immunoassay using a pair of mouse monoclonal antibodies raised against follistatin 288. The presence of sodium deoxycholate and Tween 20 in the diluent gave

results for total (free and activin-dissociated) follistatin. The assay had a detection limit of <19 pg/ml and recovery of spiked follistatin 288 from amniotic fluid, serum seminal plasma, human follicular fluid and granulosa cell conditioned medium averaged 100.7 +/- 7.5%, 89.1 +/- 5.5%, 98 +/- 4.9%, 96 +/- 7.2% and 123.9 +/- 11% respectively. The intra- and interplate coefficients of variation were < 5%. An excess of activin-A (50 ng/ml) prior to assay did not affect follistatin recovery. Inhibin-A, inhibin-B, activin-A, activin-B and activin-AB had minimal cross-reactivity (<0.3%). However, follistatin 315 had a significant cross-reaction (9.9%). Serially diluted human samples gave dose-response curves parallel to the standard. Pooled human follicular fluid contained high concentrations of follistatin (approximately 242 ng/ml). Follistatin was also found in maternal serum during pregnancy (first trimester approximately 0.8 ng/ml, third trimester approximately 2.8 ng/ml), normal male serum (approximately 0.45 ng/ml), amniotic fluid (sixteen week approximately 3.63 ng/ml, term approximately 0.89 ng/ml), seminal plasma (2.4-30 ng/ml) and human granulosa cell conditioned media (approximately 0.44 ng/ml). Serial serum samples taken throughout the menstrual cycle of ten women showed fluctuating follistatin concentrations (approximately 0.62 ng/ml) with no apparent relationship to the stage of the cycle. Interestingly, pooled serum from **postmenopausal** women appeared to have higher follistatin levels than any of the normal women (approximately 1.4 ng/ml). The possible presence in certain samples of mixtures of follistatin **isoforms** with different immunoreactivities poses major problems of interpretation in this and all other current follistatin immunoassays. Further work is needed to identify the major immunoreactive forms in different tissues and fluids. Nevertheless, the new assay has a number of advantages over previous assays and should prove a useful tool for various clinical and physiological studies.

Tags: Female; Human; Male; Pregnancy; Support, Non-U.S. Gov't

Descriptors: *Glycoproteins--analysis--AN; Amniotic Fluid--chemistry--CH; Animals; Culture Media, Conditioned--chemistry--CH; Dose-Response Relationship, Drug; Enzyme-Linked Immunosorbent Assay; Follicular Fluid --chemistry--CH; Follistatin; Glycoproteins--blood--BL; Granulosa Cells --secretion--SE; Isomerism; Menstruation--blood--BL; Mice; Mice, Inbred BALB C; **Postmenopause** --blood--BL; Regression Analysis; Semen--chemistry --CH; Sensitivity and Specificity

CAS Registry No.: 0 (Culture Media, Conditioned); 0 (Follistatin); 0 (Glycoproteins)

Record Date Created: 19980403

Record Date Completed: 19980403

8/9/40

DIALOG(R)File 155:MEDLINE(R)

13480354 PMID: 9166111

Development, validation and application of a two-site enzyme-linked immunosorbent assay for activin-AB.

Evans L W; Muttukrishna S; Knight P G; Groome N P

School of Biological and Molecular Sciences, Oxford Brookes University, UK.

Journal of endocrinology (ENGLAND) May 1997, 153 (2) p221-30, ISSN 0022-0795 Journal Code: 0375363

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Monoclonal antibodies, specific for the beta A and beta B subunits of activin, were used to develop a new two-site ELISA for activin-AB. The assay had a detection limit of 0.19 ng/ml. High concentrations of activin-AB were found in bovine, ovine and porcine follicular fluids (FF), with less in human FF (1310, 1730, 688 and 7 ng/ml respectively). Recovery of spiked activin-AB standard from human, bovine and ovine FFs and from homogenized human placental extracts averaged 91%, 115%, 115% and 94% respectively. Within-plate coefficients of variation for different

CAS Registry No.: 0 (Chorionic Gonadotropin, beta Subunit, Human); 0 (Peptide Fragments); 0 (urinary gonadotropin fragment); 9002-67-9 (Luteinizing Hormone)
Record Date Created: 20000120
Record Date Completed: 20000120

8/9/130
DIALOG(R)File 155:MEDLINE(R)
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10140163 PMID: 8033370
17 beta-oestradiol counteracts the formation of the more acidic isoforms **of follicle-stimulating hormone and luteinizing hormone after menopause.**
Wide L; Naessen T
Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.
Clinical endocrinology (ENGLAND) Jun 1994, 40 (6) p783-9, ISSN 0300-0664 Journal Code: 0346653
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
OBJECTIVE: When the gonadotrophin levels increase at midcycle, more basic **isoforms** of FSH and LH appear in the circulation. However, when these gonadotrophins increase at menopause more acidic forms appear. The present study was done to see whether chronic 17 beta-oestradiol (E2) administration to post-menopausal women could counteract the formation of the more acidic **isoforms** after the menopause. DESIGN: Serum samples were obtained from 16 post-menopausal women, mean age 70 years (range 63-84 years), 46-169 days after the subcutaneous insertion of a 20-mg E2-implant. FSH, LH and E2 in the sera were measured with fluoroimmunoassays. The median charge and the degree of charge heterogeneity of the FSH and LH **isoforms** were determined for each serum by electrophoresis in 0.1% agarose suspension. Sera from an age-matched control group were analysed in parallel. RESULTS: The E2 levels in the E2-treated women were 230-570 pmol/l, within the range expected during the mid-luteal phase of the normal menstrual cycle. The mean serum FSH and LH levels were similar to normal follicular phase FSH and LH levels (8.6 and 20.8% respectively of the control group). It was estimated that individual serum specimens from both groups contained 20-30 different **isoforms** for both FSH and LH. The median charges of the **isoforms** of FSH and LH were more basic in all the E2-treated subjects than in their corresponding untreated controls. The mean median charge for FSH was close to the values for the follicular and luteal phases and that for LH close to that for the luteal phase. In some E2-treated women the **isoforms** were even more basic with a charge similar to that at the midcycle peak. The degree of charge heterogeneity for the E2-treated group was significantly ($P < 0.001$) larger than for the controls and similar to that during the normal menstrual cycle. CONCLUSION: Chronic E2 administration to post-menopausal women counteracted the formation of more acidic **isoforms** of both FSH and LH after the menopause.
Tags: Comparative Study; Female; Human
Descriptors: Estradiol--therapeutic use--TU; *Estrogen Replacement Therapy; *Gonadotropins, Pituitary--blood--BL; * **Menopause** --blood--BL; Aged; Aged, 80 and over; Drug Implants; Electrophoresis, Agar Gel; Estradiol--blood--BL; Fluoroimmunoassay; Follicle Stimulating Hormone --blood--BL; Luteinizing Hormone--blood--BL; Middle Aged; Reference Values
CAS Registry No.: 0 (Drug Implants); 0 (Gonadotropins, Pituitary); 50-28-2 (Estradiol); 9002-67-9 (Luteinizing Hormone); 9002-68-0 (Follicle Stimulating Hormone)
Record Date Created: 19940816
Record Date Completed: 19940816

8/9/134
DIALOG(R)File 155:MEDLINE(R)
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DIALOG(R)File 155:MEDLINE(R)

13179650 PMID: 8849573

FSH isoforms : **bio and immuno-activities in post-menopausal and normal menstruating women.**

Creus S; Pellizzari E; Cigorraga S B; Campo S
Centro de Investigaciones Endocrinologicas, Hospital General de Ninos R. Gutierrez, Buenos Aires, Argentina.

Clinical endocrinology (ENGLAND) Feb 1996, 44 (2) p181-9, ISSN 0300-0664 Journal Code: 0346653
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

OBJECTIVE: The full expression of gonadotrophin biological activity depends on the gonadotrophin carbohydrate component. Our aim was to study serum FSH **isoforms** present in the follicular phase (FPS) and in the menopause (PMS) since the endocrine status may influence the structure of incorporated oligosaccharides. SUBJECTS: Ten healthy post-menopausal women (age range 53-68) not receiving any hormonal treatment and 10 healthy women (age range 20-28) in the follicular phase of their menstrual cycle were studied. MEASUREMENTS: Bio and immuno FSH-activities (Sertoli cell aromatase induction assay and RIA, respectively) were determined in separated **isoforms** after concanavalin A chromatography. Isolated **isoforms** were: UB, unbound; WB, weakly bound and FB, firmly bound to the lectin. RESULTS: PMS showed two groups of immuno and bio-active FSH **isoforms** : WB, bearing biantennary galactosylated type and FB, bearing high mannose or hybrid type oligosaccharides. Immuno and bio-active FSH were not detected in the UB fractions. WB **isoforms** represented 82 +/- 6% of the total bioactivity recovered in samples analysed individually; their B/I ratio was 0.85 +/- 0.20. FB **isoforms** were 18 +/- 6%; their B/I ratio was 3.27 +/- 0.60. Whole serum B/I ratio was 1.20 +/- 0.30. Similar results were obtained when pooled sera was analysed: WB: 77%, B/I: 0.82; FB: 23%, B/I: 3.75. Whole serum B/I in pooled samples was 1.10. FPS showed a different pattern. UB **isoforms** , bearing triantennary or bisecting oligosaccharides, were 41 +/- 3% of the total bioactivity recovered in samples analysed individually. Their B/I ratio was 0.61 +/- 0.23. WB **isoforms** were 59 +/- 3% and their B/I 0.76 +/- 0.14. FB FSH **isoforms** were not detected. The whole serum B/I ratio was 0.60 +/- 0.30. Similar results were obtained when pooled sera was analysed: WB 43%, B/I 0.42; FB 57%, B/I 0.62. Whole serum B/I in pooled samples was 0.70. CONCLUSIONS: These results show that, in normal women, circulating FSH bioactivity is associated with **isoforms** with different oligosaccharide [correction of oligosacharide] structures according to hormonal status. FSH in the follicular phase has a higher degree of branching and a more complete carbohydrate chain than the FSH secreted during the menopause.

Tags: Female; Human; Male; Support, Non-U.S. Gov't
Descriptors: Follicle Stimulating Hormone--metabolism--ME; *Follicular Phase--metabolism--ME; * **Postmenopause** --metabolism--ME; Adult; Aged; Aromatase--metabolism--ME; Biological Assay; Chromatography, Gel; Follicle Stimulating Hormone--blood--BL; Isomerism; Middle Aged; Radioimmunoassay; Sertoli Cells--drug effects--DE; Sertoli Cells--enzymology--EN
CAS Registry No.: 9002-68-0 (Follicle Stimulating Hormone)
Enzyme No.: EC 1.14.14.1 (Aromatase)

00928408

METHOD AND SYSTEM FOR DIAGNOSING ANDROPAUSE IN MALES

PROCEDE ET SYSTEME PERMETTANT DE DIAGNOSTIQUER L'ANDROPAUSE CHEZ LES HOMMES

Patent Applicant/Inventor:
ADAMS Ken, 203 Reiner Road, Toronto, Ontario M3H 2M2, CA, CA (Residence), CA (Nationality)
MACFADDEN Doug K, 79 Doncaster Avenue, Toronto, Ontario M4C 5S6, CA, CA (Residence), CA (Nationality)
Legal Representative:
BARTOSZEWICZ Lola A (et al) (agent), Sim & McBurney, 330 University Avenue, 6th Floor, Toronto, Ontario M5G 1R7, CA,

Patent and Priority Information (Country, Number, Date):
Patent: WO 200261437 A2-A3 **20020808** (WO 0261437)
Application: WO 2002CA119 20020130 (PCT/WO CA0200119)
Priority Application: US 2001264732 20010130
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ
EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR
LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI
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(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 6242

0005722524 **IMAGE Available
Derwent Accession: 2002-619284
US 20040137520
Method and system for diagnosing andropause in males
Inventor: Adams, Ken, INV
MacFadden, Doug, INV
Correspondence Address: DENISE M GLASSMEYER YOUNG & BASILE, P.C., 3001 W. BIG BEAVER RD., SUITE 624, TROY, MI, 48084-2813, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20040137520	A1	20040715	US 2004470819	20040108
PCT				WO 2002CA119	20020130

Fulltext Word Count: 8057

... 20040715

Summary of the Invention:

...the diagnosis of androgen decline in males now commonly referred to as andropause or male **menopause** . In particular, the present invention relates to a diagnostic method, **device** and test kit for indicating andropausal status and/or reduced gonadal function in males...

...to as male menopause, is defined as a progressive decline in the production of androgenic **hormones** which commonly occurs in ageing adult males. The age of onset of andropause is very...40, and the incidence increases with each decade of life. Associated with declines in androgenic **hormone** production are declines in male gonadal function which tend to develop very gradually over many...

...which a decline in ovarian function results in a corresponding decline in the levels of **estrogen** .

[...

...In both andropause and female menopause the decline in biologically active levels of the sex **hormones** causes the clinical signs and symptoms associated with these two conditions. The declining levels of gonadal **hormones** are a direct result of a progressive reduction of

Record Date Created: 19970102
Record Date Completed: 19970102

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DIALOG(R)File 155:MEDLINE(R)
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12706091 PMID: 7628088
Immunometric assays of luteinizing hormone (LH): differences in recognition of plasma LH by anti-intact and beta-subunit-specific antibodies in various physiological and pathophysiological situations.
Mitchell R; Hollis S; Crowley V; McLoughlin J; Peers N; Robertson W R
University of Manchester Department of Medicine, Hope Hospital, Salford, UK.
Clinical chemistry (UNITED STATES) Aug 1995, 41 (8 Pt 1) p1139-45, ISSN 0009-9147 Journal Code: 9421549
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Restricted immunoreactivity of plasma luteinizing hormone (LH) has been described in some subjects when assayed with certain methods involving antibodies against intact LH. We have compared the performance of the Amerlite LH-30 (A) and Delfia LHSpec (D) assays (which include anti-intact and beta-specific antibodies, respectively) in normal and pathological conditions. As shown previously, results of the two systems were highly correlated with each other and, as we show here, with those of a bioassay. We found eight outliers (results outside the 95% confidence interval of the regression) among 427 samples studied from 121 subjects. Of the outliers, five had Delfia results in a range (< 1 IU/L) that was associated with poor assay precision for that assay, and the ratios of their values by both methods (A:D ratios) were very low. This ratio was affected by endocrine status, e.g., was lower in **postmenopausal** women than in premenopausal controls, and varied intraindividually within the same menstrual cycle. The restricted immunoreactivity described previously for assays involving anti-intact LH antibodies may, in part, reflect these differences, which, in turn, may reflect the presence of **isoforms** (e.g., **glycoforms**) that are differentially recognized by assays that have different antibody configurations.
Tags: Comparative Study; Female; Human; Male; Support, Non-U.S. Gov't
Descriptors: *Antibodies--immunology--IM; *Immunoassay; *Luteinizing Hormone--blood--BL; *Luteinizing Hormone--immunology--IM; Antibody Specificity; Fertilization in Vitro; Immunoassay--standards--ST; Immunoassay--statistics and numerical data--SN; Polycystic Ovary Syndrome; **Postmenopause** --blood--BL; Reagent Kits, Diagnostic--standards--ST; Reagent Kits, Diagnostic--statistics and numerical data--SN
CAS Registry No.: 0 (Antibodies); 0 (Reagent Kits, Diagnostic); 9002-67-9 (Luteinizing Hormone)
Record Date Created: 19950907
Record Date Completed: 19950907

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DIALOG(R)File 155:MEDLINE(R)
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12593467 PMID: 7706979
Inhibin forms in human plasma.
Robertson D M; Sullivan J; Watson M; Cahir N
Prince Henry's Institute of Medical Research, Monash Medical Centre, Clayton, Victoria, Australia.
Journal of endocrinology (ENGLAND) Feb 1995, 144 (2) p261-9, ISSN 0022-0795 Journal Code: 0375363
Document type: Journal Article
Languages: ENGLISH

• Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

In order to identify the molecular weight forms of bioactive and immunoactive inhibin in human plasma, plasma/serum was sequentially fractionated by immunoaffinity chromatography (using immobilised inhibin alpha subunit antiserum), reversed phase HPLC and preparative SDS-PAGE. The electroeluted gel fractions were assayed for inhibin in vitro bioactivity and immunoactivity, the latter by RIA. Initial experiments examined human follicular fluid as an inhibin-rich source. Bioactive and immunoactive fractions of 30, 35, 53, 65 and approximately 120 kDa were identified in addition to bio-inactive, immunoactive fractions of 26 kDa and 32 kDa. These molecular weights correspond to those of known inhibin forms and are attributed to differing degrees of **glycosylation** of the inhibin alpha subunit and variable processing of the alpha and beta inhibin subunits. Fractionation of male plasma pools revealed the presence of higher molecular weight immunoactive forms (55-120 kDa) as well as 28-31 kDa forms although the molecular weight distribution of activity between pools varied. To assess if the molecular weight pattern was modified by storage and/or subsequent fractionation, protease inhibitors were added initially to plasma and fractionated as above. The molecular weight distribution of immunoactivity was largely unaffected by the treatment, indicating that minimal processing had occurred. **Postmenopausal** serum itself showed low to undetectable activity. The addition of recombinant human 31 kDa inhibin to **postmenopausal** serum resulted in a molecular weight profile of inhibin immunoactivity consistent with the presence of 31 kDa inhibin. Fractionation of a serum pool from women undergoing gonadotrophin stimulation, in which inhibin levels were elevated, showed a range of bioactive and immunoactive inhibin forms over the 30-120 kDa range.(ABSTRACT TRUNCATED AT 250 WORDS)

Tags: Female; Human; Male; Support, Non-U.S. Gov't
Descriptors: *Inhibins--blood--BL; Chromatography, Affinity; Chromatography, High Pressure Liquid; Electrophoresis, Polyacrylamide Gel; Inhibins--chemistry--CH; Molecular Weight; Radioimmunoassay
CAS Registry No.: 57285-09-3 (Inhibins)
Record Date Created: 19950510
Record Date Completed: 19950510

8/9/55
DIALOG(R)File 155:MEDLINE(R)

12549666 PMID: 7890049
Pharmacokinetics of follicle-stimulating hormone: clinical significance.
Ben-Rafael Z; Levy T; Schoemaker J
Golda Meir Medical Center, Petah Tiqva, Israel.
Fertility and sterility (UNITED STATES) Apr 1995, 63 (4) p689-700, ISSN 0015-0282 Journal Code: 0372772
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

OBJECTIVE: To review studies that examine the pharmacokinetics and pharmacodynamics of endogenous, as well as several exogenous FSH preparations. DESIGN: Related studies were identified through a computerized bibliographic search. PATIENTS: Initial pharmacodynamic studies were done in animal models and in women and men with either hypogonadotropic hypogonadism or suppressed hypothalamic-pituitary-gonadal axis. More recent studies evaluated FSH pharmacokinetics during ovulation induction treatment in women with normal ovulatory cycles or polycystic ovarian syndrome. RESULTS: Various types of FSH exist according to their sialic acid content. High estrogen levels induce the secretion of less **sialylated** molecules with higher receptor affinity and an increased clearance rate. It appears that there is a threshold FSH level that should be reached to achieve an ovarian response. A very narrow range exists

10576824 96389001 PMID: 8796333

European collaborative study of LH assay : 3. relationship of immunological reactivity, biological activity and charge of human luteinizing hormone.

Niccoli P; Costagliola S; Patricot M C; Mallet B; Benahmed M; Carayon P
Laboratoire de Biochimie Endocrinienne et Metabolique, Unite 38 INSERM, Faculte de Medecine, Marseille, France.

Journal of endocrinological investigation (ITALY) May 1996; 19 (5) p260-7, ISSN 0391-4097 Journal Code: 7806594
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

This report describes the results of the third part of the collaborative study organized by a working group sponsored by the Community Bureau of Reference of the European Community Commission. The aim of the present work was to establish the link between immunoreactivity and biological activity of human LH, thus allowing to determine the antigenic domains of the molecule involved in the induction of the biological effect. The relationship between immunoreactivity and electric charge of hLH was also studied. This work allowed to further apprehend hLH isomorphism and its role in discrepancies observed among hLH **assays** and clinical status. It also made the feasibility of measuring biologically active **isoforms** by an immunological **method** to be assessed. The effect of 36 mAb with known epitopic specificity, was evaluated on both hLH binding to rat membrane receptor and hLH induced production of testosterone by porcine Leydig cells. All the epitopes located on the beta subunit were found to be strongly involved in the biological activity whereas 4/9 and 10/18 epitopes present on the alpha subunit or specific for the holomolecule respectively appeared weakly involved. **Assaying** biological hLH using immunological **method** would require that mAb specific for all the epitopes involved in the receptor activation be tested, and thus appears presently unsuitable for routine clinical evaluation. In the previous work some LH **immunoassays** were found to underestimate LH concentrations (J. Endocrinol. Invest 1994, 17: 397-406 and 407-416). The mAb used in liquid phase in these kits were found in the present work to be directed against the domains of LH weakly involved in the activation of the receptor and would suggest that bioactive LH would be misevaluated by these kits. The immunoreactivity of hLH **isoforms** **separated** by isoelectric focusing (IEF) in liquid phase was also determined. IEF allowed to **separate** three groups of hLH **isoforms** but none of them exhibited a specific discriminating pattern of immunoreactivity when they were tested against a panel of mAb. It suggests that, in our experimental conditions, the electric charge and the immunoreactivity of hLH were not closely linked.

Tags: Animal; Human; Male

United States Patent [19]

Canfield et al.

[11] **Patent Number: 4,514,505**

[45] **Date of Patent: Apr. 30, 1985**

[54] **MONOCLONAL ANTIBODY MIXTURES AND USE THEREOF FOR ENHANCED SENSITIVITY IMMUNOASSAYS**

[75] Inventors: **Robert E. Canfield; Paul H. Ehrlich**, both of New York, N.Y.; **William R. Moyle**, Piscataway, N.J.

[73] Assignee: **The Trustees of Columbia University in the City of New York**, New York, N.Y.

[21] Appl. No.: **380,959**

[22] Filed: **May 21, 1982**

[51] **Int. Cl.**[3] **G01N 33/54**; G01N 33/74; G01N 33/76

[52] **U.S. Cl.** **436/500**; 436/510; 436/518; 436/536; 436/542; 436/548; 436/804; 436/817; 436/818; 435/7; 935/110

[58] **Field of Search** ..... 436/548, 518, 540, 547, 436/65, 86, 811, 814, 815, 817, 818, 500, 510, 514–516, 536, 542, 804; 424/1, 1.5; 435/4, 7, 68, 172, 948; 260/112 R; 935/110

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Primary Examiner—Benjamin R. Padgett
Assistant Examiner—M. Moskowitz
Attorney, Agent, or Firm—John P. White

[57] **ABSTRACT**

Mixtures of monoclonal antibodies which contain effective assaying amounts of each of at least two monoclonal antibodies that bind to different antigenic sites on the antigen and are capable under appropriate conditions of binding simultaneously to an antigen are useful in enhanced sensitivity assays for the antigen. By utilizing such mixtures in diagnostic assays for important antigens such as the polypeptide human chorionic gonadotropin enhanced sensitivity can be achieved as compared with assays employing individual monoclonal antibodies.

16 Claims, 7 Drawing Figures

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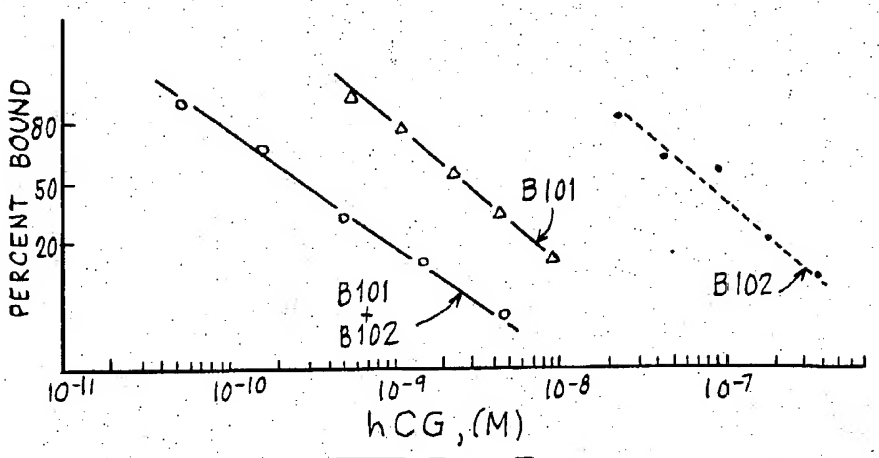

PERCENT BOUND
80
50
20
B101
B101
+
B102
B102
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
hCG, (M)

Fig. 1.

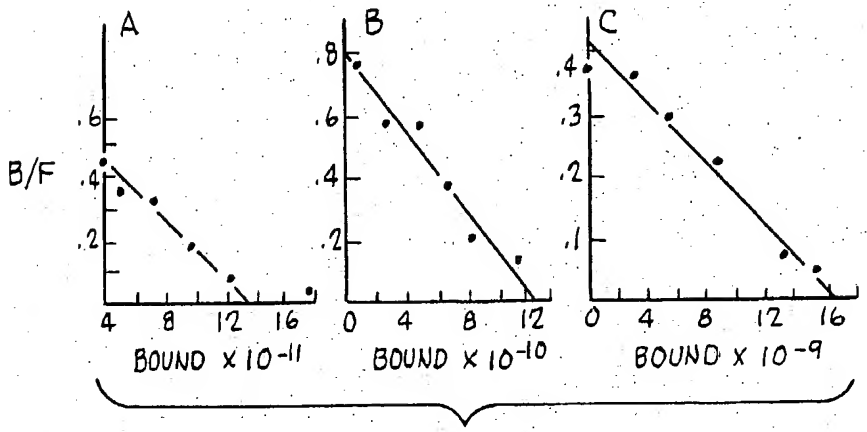

A
B
C
B/F
.8
.6
.4
.2
.4
.3
.2
.1
4 8 12 16
0 4 8 12
0 4 8 12 16
BOUND X 10^{-11}
BOUND X 10^{-10}
BOUND X 10^{-9}

Fig. 2.

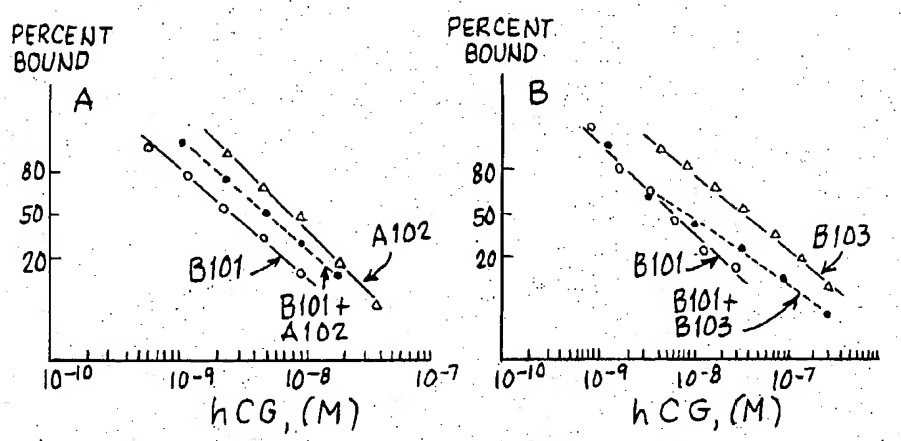

PERCENT BOUND
A
80
50
20
B101
A102
B101+ A102
10^{-10}
10^{-9}
10^{-8}
10^{-7}
hCG, (M)
PERCENT BOUND
B
80
50
20
B101
B103
B101+ B103
10^{-10}
10^{-9}
10^{-8}
10^{-7}
hCG, (M)

Fig. 3.

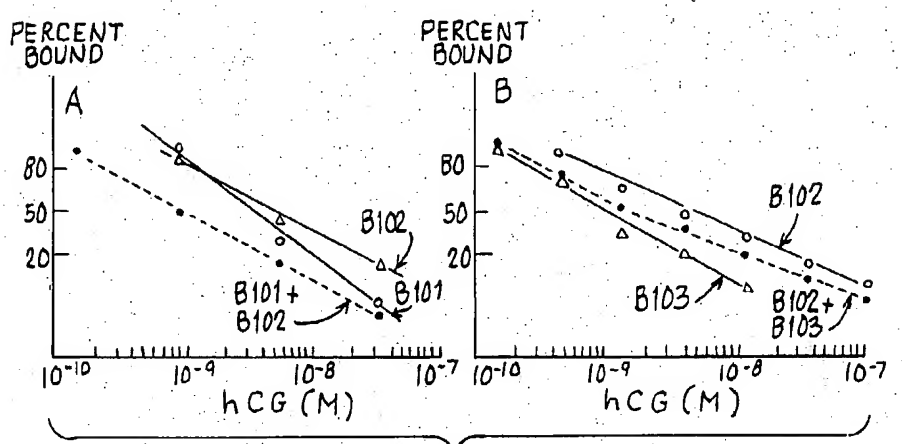

PERCENT BOUND
A
80
50
20
B102
B101
B101+ B102
10^{-10}
10^{-9}
10^{-8}
10^{-7}
hCG (M)
PERCENT BOUND
B
80
50
20
B102
B103
B102+ B103
10^{-10}
10^{-9}
10^{-8}
10^{-7}
hCG (M)

Fig. 4.

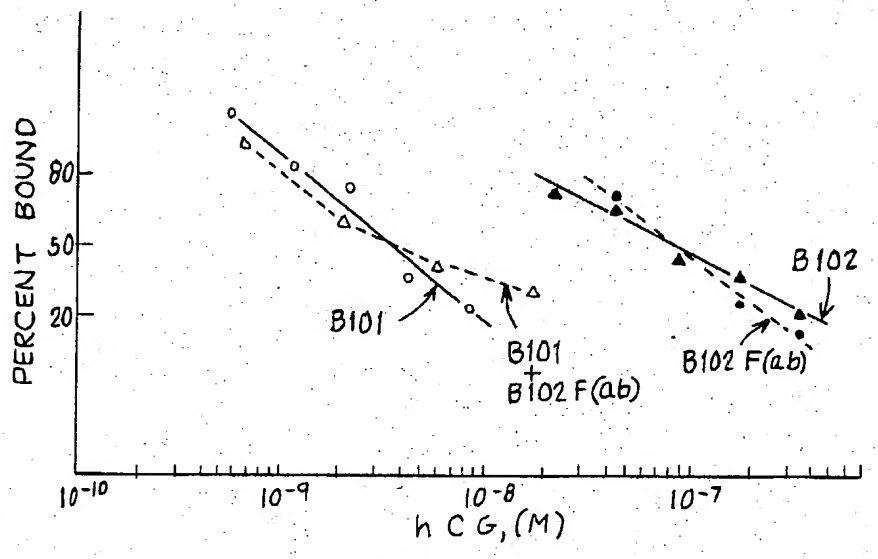

PERCENT BOUND
80
50
20
B101
B101
+
B102 F(ab)
B102
B102 F(ab)
10-10
10-9
10-8
10-7
h C G, (M)

Fig. 5.

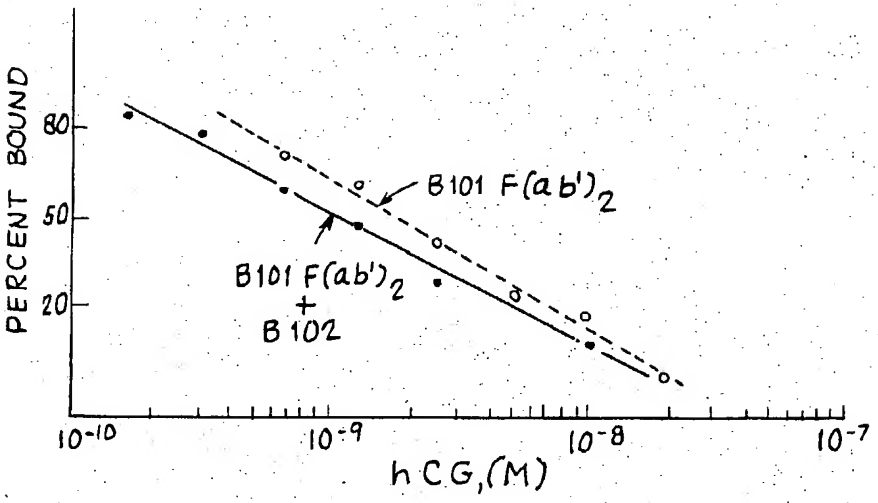
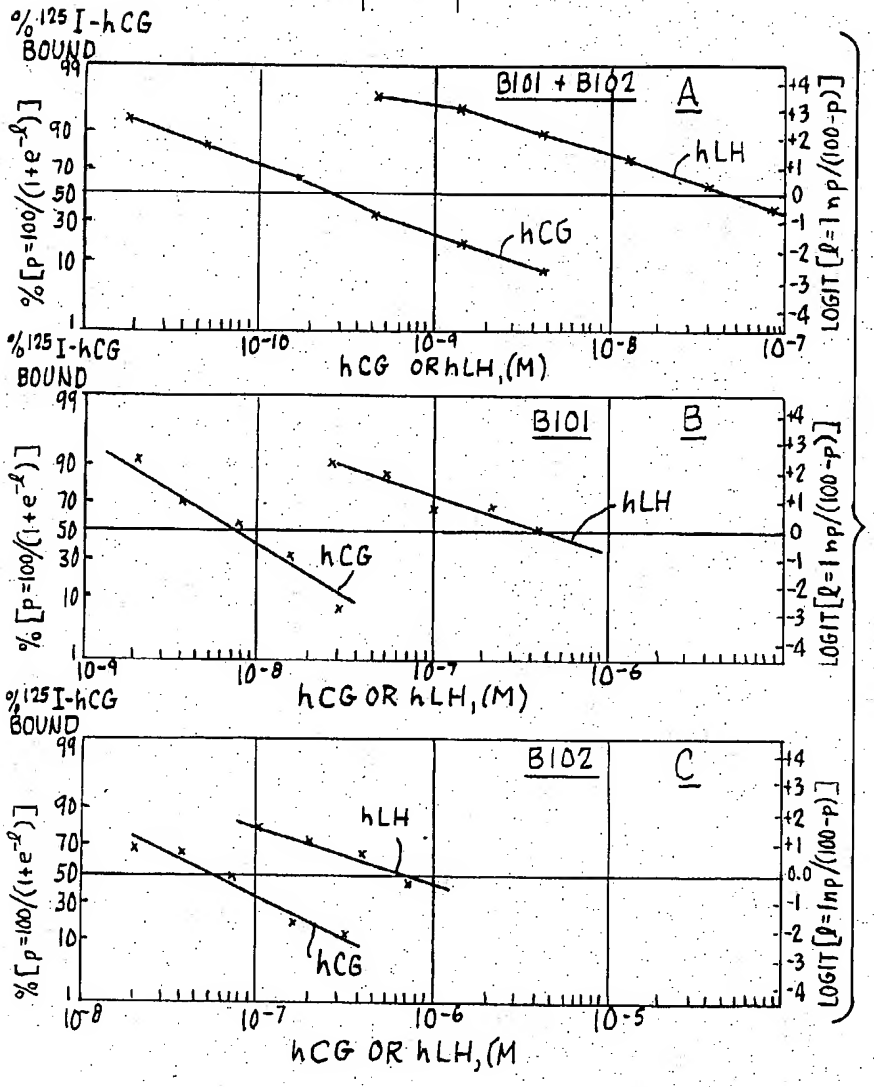

PERCENT BOUND
80
50
20
B101 F(ab')2
B101 F(ab')2
+
B102
10-10
10-9
10-8
10-7
h CG, (M)

Fig. 6.

Fig. 7.


% 125 I-hCG BOUND
B101 + B102
A
hLH
hCG
% [p=100/(1+e^-l)]
LOGIT [l=1 np/(100-p)]
hCG OR hLH, (M)
B101
B
hLH
hCG
B102
C
hLH
hCG

MONOCLONAL ANTIBODY MIXTURES AND USE THEREOF FOR ENHANCED SENSITIVITY IMMUNOASSAYS

The invention described herein was made in the course of work under grants numbered HD-13496, HD-15454 and CA-26636 from the National Institutes of Health, United States Department of Health and Human Services.

BACKGROUND OF THE INVENTION

Development of the hybridoma technology [Kohler, G., and C. Milstein, (1975) Nature 256:495 and; Goding, J. W., (1980) Immunological Methods 39:285] has provided immunoglobulin reagents which bind to only one antigenic site. Although these reagents have found widespread use as biochemical and immunological tools, their usefulness in radioimmunoassay has frequently been limited by their lower affinity for antigen compared with that of serum antibodies [Goding, J. W., (1980) Immunological Methods 39:285]. In principle, the affinity of monoclonal antibodies could be enhanced by more stringent hybridoma selection procedures. The production of monoclonal antibodies has also enabled investigators to dissect the humoral immune response into its pure components [Staines, N. A. and A. M Lew (1980), Immunology 40:287]. This will eventually result in a more comprehensive understanding of the role of the individual antibody, especially with regard to the possibility that an antiserum may have different characteristics than the sum of its individual antibodies.

During the course of a systematic assessment of the immunochemistry of human chorionic gonadotropin (hCG), including the relative orientation of different epitopes of this molecule and the effect of several monoclonal antibodies on the hormone-receptor interaction, it has been observed that the apparent affinity of mixtures of certain monoclonal antibodies is enhanced relative to the affinity of the individual antibodies.

SUMMARY OF THE INVENTION

A mixture of monoclonal antibodies which includes an effective assaying amount of each of at least two monoclonal antibodies that bind to different antigenic sites on an antigen and are capable of doing so under appropriate conditions is useful in an enhanced sensitivity assay for the antigen.

Such mixtures are particularly useful in assays for antigens having multiple epitopes, e.g. polypeptides such as human chorionic gonadotropin, follicle stimulating hormone, thyroid stimulating hormone or luteinizing hormone where the different antigenic sites on the antigen are different amino acid sequences contained therein. By employing such mixtures in assays for antigens of clinical interest one can obtain enhanced sensitivity as compared with the sensitivity obtained with the individual monoclonal antibodies.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Radioimmmunoassay for human chorionic gonadotropin (hCG) by double antibody liquid phase assay. The amount of labeled hCG bound in the absence of unlabeled inhibitor was approximately the same for each antibody or antibody mixture. This was achieved by appropriate dilution of each antibody solution or mixture. Rabbit anti-mouse IgG was the second antibody. The ED_{50} plus/minus the standard deviation was calculated for each antibody or mixture. The ED_{50} for the mixture of B101 and B102 is $2.44\pm0.74\times10^{-10}$M, antibody B101 is $2.88\pm0.74\times10^{-9}$M, and antibody B102 is $1.058\pm0.995\times10^{-7}$M.

FIG. 2: Scatchard analysis of the binding to hCG of A, a mixture of antibodies B101 and B102; B, antibody B101 and C, antibody B102. Slopes of the lines (therefore, the equilibrium binding constants) are A: 5.4×10^{9}; B: 5.1×10^{8}; and C: 1.9×10^{7}.

FIG. 3A: Radioimmunoassay for hCG by double antibody liquid phase assay. The procedure was the same as for FIG. 1. The ED_{50} for antibody A102 is $8.52\pm2.94\times10^{-9}$M, antibody B101 is $2.88\pm0.74\times10^{-9}$M, and mixture of antibodies B101 and A102 is $4.66\pm1.99\times10^{-9}$M.

FIG. 3B: Radioimmunoassay for hCG by double antibody liquid phase assay with antibodies B101, B103, and a mixture of B101 and B103. The procedure was the same as in FIG. 1.

FIG. 4: Radioimmunoassay for hCG by solid phase assay. A: Antibodies B101, B102 and a mixture of B101 and B102. B: Antibodies B102, B103 and a mixture of B102 and B103.

FIG. 5: Radioimmunoassay for hCG by double antibody liquid phase assay with papain digested B102. The procedure was the same as for FIG. 1 except that the second antibody used for precipitation of the antibody-antigen complex was goat anti-mouse $F(ab')_2$. The increase in affinity of the mixture was not affected by this change of second antibody (results not shown).

FIG. 6: Radioimmunoassay for hCG by double antibody liquid phase assay with B101-$F(ab')_2$. The procedure was the same as for FIG. 5.

FIG. 7: Logit inhibition curves of A: a mixture of B101 and B102, B: antibody B101 and C: antibody B102 with hCG and with human luteinizing hormone (a hormone with a very similar structure).

DETAILED DESCRIPTION OF THE INVENTION

It has been observed that mixing monoclonal antibodies directed against various epitopes of human chorionic gonadotropin can increase the sensitivity of antigen binding assays. Depending on the antibody pair chosen, the affinity of the mixture can be as much as 10-fold higher than that of the monoclonal antibodies assayed separately. This increased affinity can be detected in both a solid phase assay and a liquid phase double antibody radioimmunoassay. The mechanism for the increase in affinity depends on the formation of a multicomponent complex. Mixing two antibodies which cannot bind simultaneously does not result in enhanced affinity, but combining pairs which can bind at the same time under appropriate conditions results in higher sensitivity in an antigen binding assay. If one of the antibodies of a pair which results in enhanced affinity upon mixing is replaced by its F(ab) fragment, the enhancement is no longer detectable, indicating that it is unlikely that the enhancement is due to an allosteric effect. While the $F(ab')_2$ fragment shows some enhancement when mixed with another antibody, it is not as effective as the intact antibody.

Based upon these discoveries and observations, the present invention provides mixtures of monoclonal antibodies useful in an enhanced sensitivity assay for an antigen. The antibodies useful in the practices of the inventions are also characterized by the fact that they are capable of binding to the antigen both under normal

nondenaturing conditions and under conditions described hereinafter where antibodies otherwise capable of binding to the antigen are unable to do so.

The invention described herein may in principle involve more than two monoclonal antibodies. Mixtures containing three, four or more antibodies each of which binds to a different antigenic site on the antigen may be particularly useful in assays for large molecules such as polypeptides having numerous subunits. However, for illustrative purposes the invention will be described by reference to mixtures which contain two such antibodies.

Although various types of antigens could be detected in assays employing mixtures of suitable monoclonal antibodies, the invention is particularly suited for detection of polypeptide antigens where the different antigenic sites are different and distinct amino acid sequences contained within the polypeptide. Examples of antigens of this type are the sequence related hormones human chorionic gonadotropin (hCG), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and luteinizing hormone (LH).

To be effective in assay, each monoclonal antibody of the assay mixture should be present in an amount sufficient to permit significant binding to the antigen, that is, above about 10 percent of the antigen to be simultaneously bound to the antibodies. Preferably the amount of antigen bound should be higher, for example an amount in the range from about 20 to about 80 percent or higher.

In order to obtain such amounts of bound antigen, the precise amount of each antibody may vary widely depending upon its affinity for the antigen so that lesser amounts of antibodies having higher affinities are required than of antibodies having lower affinities.

The amounts of each of the monoclonal antibodies relative to one another will also vary over a wide range. For a two antibody mixture, the amount may vary from about 10^6:1 to 1:10^6. However, based upon the binding constants most likely to characterize the monoclonal antibodies of interest the range might preferably be from about 10^2:1 to 1:10^2. In general, the preferred amount of each antibody relative to the amount of other antibodies will be substantially the same as the ratio of the binding constants of the antibodies to the antigen. Thus, if the binding constants of monoclonal antibodies A and B are 10^{-9} and 10^{-7}, respectively, the relative amounts of A and B will be about $10^{-9}/10^{-7}=10^{-2}$, that is, 1 molecule of A for each 100 molecules of B.

In the case of polypeptide antigens which contain subunits such as the α and β subunits of human chorionic gonadotropin, mixtures of monoclonal antibodies in which each of the antibodies binds to an antigenic site on a different subunit are more likely to provide enhanced sensitivity in assays employing them, possibly because they are more likely to be capable of simultaneously binding to the antigen under appropriate conditions.

The mixtures may be used in assays in different forms. Thus, they may be used in solid form absorbed onto a solid matrix or in liquid form dissolved in a suitable buffered solution.

The mixtures can be employed in conventional immunoassays, including radioimmunoassays, to determine qualitatively and quantitatively the presence of antigens in samples of interest. In such methods the samples are contacted with the mixture under suitable conditions permitting formation of a detectable complex between the mixture and the antigen. One specific application would involve detection of human chlorionic gonadotropic as a test for pregnancy.

To assist in an understanding of the invention the results of a series of experiments follow. Of course, these experiments are intended merely to illustrate the invention and should not in any way be construed as limiting its scope as defined by the claims which follow thereafter.

EXPERIMENTAL DETAILS

Materials and Methods

Production of Monoclonal Antibodies: Balb/c mice were immunized monthly with an intraperitoneal injection of hCG subunits in complete Freund's adjuvant for several months according to the method of Wands and Zurawski [Wands, J. R., and V. R. Zurawski, (1981) Gastroenterology 80:225]. Three days before the spleen was excised, additional antigen was injected (50 μg, I.V., in saline).

The spleen cells were fused with P3-NS1/1-Ag4-1 myeloma cells and hybridoma cells were isolated using published procedures [Wands, J. R., and V. R. Zurawski, (1981) Gastroenterology 80:225; Marshak-Rothstein, A., et al., (1979) J. Immunol. 122:2491]. Antibodies used for subsequent studies were isolated from hybridoma cell lines which were cloned twice by limiting dilution on Balb/c 3T3 monolayers. The antibodies are named with a letter and three numbers. The letter B indicates the antibody is specific for the β subunit of hCG and A indicates the antibody binds the α subunit.

Cell supernatant, partially purified, or purified antibody, or both were used. To obtain partially purified antibody the hybridoma cells were grown in serum-free medium containing 2 mg/ml bovine serum albumin and the supernatant dialyzed against 0.05M ammonium bicarbonate. After lyophilization, the powder was reconstituted in the desired volume of 0.3M potassium phosphate buffer, pH 7.5. To obtain purified antibody the same protocol was followed (except the medium contained 0.1 mg/ml bovine serum albumin) and the albumin removed with a DEAE Affi-Gel Blue column (Bio-Rad Laboratories).

Double Antibody Radioimmunoassay

50 μl ^{125}I-hCG and 50 μl of unlabeled hCG (both in 1 percent horse serum, 99 percent phosphate buffered saline) were mixed with 100 μl 0.3M potassium phosphate (pH 7.5). Subsequently, 100 μl antibody (diluted in 1 percent horse serum) were added, the tubes incubated one hour at 37° C. followed by 18 hours at 5° C., and the complex precipitated by adding 10 μl of 50 percent normal mouse serum (in phosphate buffered saline) and an appropriate amount of rabbit anti-mouse IgG or goat anti-mouse F(ab')$_2$. Once precipitation was complete (i.e. 10 minutes at 37° C. and then 1 hour at room temperature), the precipitate was sedimented and counted.

Solid Phase Radioimmunoassay

To coat plastic microtiter wells with antibody, 50 μl purified antibody was incubated for 18 hours at 5° C. in a Cooke microtiter plate ("U" wells) and excess nonbound antibody removed by washing the wells three times with distilled water. To saturate all the plastic sites which would bind proteins, the plates were treated with 10 percent γ-G free horse serum (90 percent phos-

phate buffered saline) for 2 hours and the excess removed with distilled water. HCG binding was quantified by adding 50 μl ^{125}I-hCG in 1 percent horse serum containing varying amounts of hCG. After an incubation of 18 hours at 5° C., excess radioactivity was removed, the plates washed with distilled water and the wells counted.

Sandwich Assay

50 μl containing at least 30 μg/ml of monoclonal antibody were added to the 96-well microtiter plates to permit the antibody to adsorb to the surface of the plastic. After 4 hours at 37° C. the solution was removed and the plates immersed in 150 mM NaCl solution containing 1 mg/ml bovine serum albumin (BSA-saline) to fill remaining nonspecific adsorption sites on the plastic surface. To saturate the antibodies with hCG, the hormone (1 μg in 50 μl BSA-saline) was added for 2 hours to each microtiter well. Under these conditions most of the hCG which became insolubilized was bound to antibody adsorbed to the plastic. Excess hCG was removed by washing the plate in BSA-saline solution. After addition of radiolabeled antibody (50,000–100,000 cpm in BSA-saline solution for 2 hours at room temperature), the non-bound label was removed by washing the plate in BSA-saline, the microtiter plates cut apart with scissors, and the radioactivity adsorbed to each well was measured.

Other Materials and Methods

Digestion of antibody B102 was performed by methods previously described [Edelman, G. M., and J. J. Marchalonis, (1967) Methods in Immunology and Immunochemistry, Vol. I, pp. 405, Academic Press, New York]. Sodium dodecyl sulfate ($NaDodSO_4$) polyacrylamide gel electrophoresis [Weber, K., and M. Osborn, (1969) J. Biol. Chem. 244:4406] of the digestion products indicated that essentially no complete heavy chain remained. The $F(ab')_2$ fragment of antibody B101 was prepared by pepsin digestion [Gorini, G., et al., (1969) J. Immunol. 103:1132], except that the amount of pepsin was 2½ percent by weight of the amount of antibody. Antibody which remained undigested was removed by incubation with Protein A-Sepharose (Pharmacia Fine Chemicals) at pH 8.1 and centrifugation of the Protein A-Sepharose-antibody complex. Conditions were similar to those employed by Ey, et al. [Ey, P. L., et al., (1978) Immunochemistry 15:429] except that a batch process instead of column chromatography was employed. $NaDodSO_4$ polyacrylamide gel electrohoresis in the absence of reducing agents revealed only one major band at a molecular weight of 105,000. The concentration of hCG was determined by the optical density at 280 nm and amino acid analysis. Chloramine-T was used to iodinate hCG according to the procedure described by Greenwood, et al. [Greenwood, F., et al., (1963) Biochem. J. 89:114].

Results

Radioimmunoassays

The ability of hCG to inhibit the binding of radioiodinated hCG to monoclonal antibodies B101, B102, and a mixture of B101 and B102 was compared (FIG. 1). HCG was 20-fold more active in inhibiting binding of radiolabel when the mixture was employed. Scatchard analysis of the data showed a similar increase in affinity of the mixture over B101 alone and a much larger increase over B102 alone (FIG. 2). The greater affinity enabled us to dilute a 1:1 mixture of the two antibodies 9-fold and chieve the same amount of tracer binding as the separate antibodies. These results were highly reproducible, as shown in Table I. While the data are not presented here, the ability of two antibodies to show this cooperative effect is not limited to pairs of antibodies which bind the same subunit since A102 and B102 also have this cooperative interaction. Other combinations of antibodies did not produce the same result. Thus, a similar cooperative effect between B101 and A102 (FIG. 3A) or between B101 and B103 (FIG. 3B) was not observed.

TABLE 1

Statistical Analysis of the Affinity of Antibodies B101 and B102 Compared with a Mixture of Antibodies B101 plus B102

Experiment	Antibody or Mixture	Keq* (L/nMole)	Enhancement Factor**	P
1	B101	0.51 ± 0.02	10.6	<.01
	B102	0.019 ± 0.002		
	B101 + B102	5.4 ± 0.5		
2	B101	0.41 ± 0.09	7.3	<.01
	B102	0.018 ± 0.002		
	B101 + B102	3.0 ± 0.2		
3	B101	0.88 ± 0.08	5.7	<.01
	B101 + B102	5.0 ± 0.07		
4	B101	0.81 ± 0.11	6.0	<.01
	B101 + B102	5.5 ± 1.2		

*Determined using Scatchard plots [Scatchard, G., (1949) Ann. N.Y. Acad. Sci. 51:660.]

**Keq for a mixture of B101 and B102 divided by Keq for B101. For the purposes of calculating the enhancement, the affinity of the mixture is compared to the affinity of the antibody with the higher affinity.

Mixtures of B101 and A102 (or B101 and B103) gave inhibition curves intermediate between those antibodies. Similar results are apparent with a solid phase radioimmunoassay (FIG. 4). A mixture of B101 and B102 was observed to have a higher affinity for hCG than either of the antibodies separately, although the difference between the mixture and antibody B101 was only about 4-fold. The combination of antibody B102 and B103 did not result in an increase in affinity.

Sandwich Assays

In order to obtain data bearing on the mechanism of the affinity change, the ability of the antibodies to bind the different sites on hCG was measured using sandwich assays (Table II). If unlabeled and radiolabeled antibody bind to the same site, the unlabeled antibody will inhibit binding of the radiolabel. Conversely, if both bind to different sites, a large amount of radiolabel will be bound to the plastic. Labeled and unlabeled B101 cannot bind the antigen at the same time; therefore, little ratiolabel was observed bound to the plastic. Similar results were observed using labeled and unlabeled B102. Antibodies B102 and B103 bound to hCG at a site remote from that for B101 since both allowed binding of B101 to the plastic. Further, labeled B102 bound to hCG attached to unlabeled B101. In contrast, antibody A102 prevented binding of radioactive B101 indicating that A102 and B101 cannot bind simultaneously to hCG. Similarly, it was found that antibodies B102 and B103 cannot bind simultaneously. Thus, it appeared that simultaneous binding of the antibodies on two different hCG sites was necessary but not sufficient for cooperative interactions.

Effect of Antibody Fragments

To study the influence of the structure of the antibodies on cooperativity, fragments of the monoclonal antibodies were prepared. The capability of antibody B102 to enhance the affinity of B101 was eliminated when antibody B102 was digested with papain (FIG. 5) although papain digestion of B102 had no detectable effect on the affinity of this antibody for hCG. This indicated that either the F(c) region or the bivalency of the antibody was necessary for enhanced affinity. The bivalent $F(ab')_2$ results in some enhancement of binding affinity but it is not as effective as the intact antibody (FIG. 6 and Table III).

TABLE II

Sandwich Assays Using Pairs of Monoclonal Antibodies

	Unlabeled Antibody			
Labeled Antibody	B101	B102	B103	A102
B101	17	9438*	11128*	415
B102	5136*	−202	144	5453*

*$p < .001$; all the others are not significantly different from zero.

Values are cpm (means of triplicate determinations) of antibody specifically bound to hCG-unlabelled antibody complexes adsorbed to the surface of the plastic microtiter plates. Radioactivity bound nonspecificaly (i.e. that which became bound to the plastic in the presence of a monoclonal antibody which does not bind hCG) was subtracted.

TABLE III

Statistical Analysis of the Affinity of B101 $F(ab')_2$ Compared with the Affinity of B101 $F(ab')_2$ + B102

Experiment	Antibody or Mixture	Keq* (L/nMole)	Enhancement Factor**	P
1	B101 $F(ab')_2$	1.61 ± 0.18***	1.5	<.1
	B101 $F(ab')_2$ + B102	2.4 ± 0.4		
2	B101 $F(ab')_2$	0.77 ± 0.11	2.2	<.01
	B101 $F(ab')_2$ + B102	1.7 ± 0.1	2.2	<.01
3	B101 $F(ab')_2$	0.97 ± 0.17	2.0	<.01
	B101 $F(ab')_2$ + B102	1.9 ± 0.2	2.0	<.01
4	B101 $F(ab')_2$	0.72 ± 0.06	6.4	<.01
	B101 $F(ab')_2$ + B102	4.6 ± 0.8		

*Determined using Scatchard plots [Scatchard, G., (1949) Ann. N.Y. Acad. Sci. 52:660.]
**Keq for a mixture of B101 $F(ab')_2$ and B102 divided by Keq for B101.
***Keq values for B101 $F(ab')_2$ are not significantly different than Keq for B101 shown in Table I.

DISCUSSION

Mixtures of monoclonal antibodies may have significantly different properties than the individual components. It has been shown that the affinity of a mixture of hybridoma antibodies can be enhanced. Other properties of monoclonal antibodies have previously been improved by utilizing more than one hybridoma antibody. Haber, et al. [Haber, E. et al., (1980) "Resolving antigenic sites and purifying proteins with monoclonal antibodies", Monoclonal Antibodies in Endocrine Research, Raven Press] developed a radioimmunometric assay with two monoclonal antibodies which had greater specificity than an assay employing either antibody separately. Howard, et al. [Howard, J. C., et al., (1979) Immunological Rev. 47:139] showed that combinations of monoclonal antibodies can be synergistic in lysing red blood cells. Although some of the advantages of monoclonal antibodies may be lost upon mixing, such as the ability to react with a single determinant, many advantages may result in producing what is, in effect, a synthetic antiserum. These results may also have implications for the control of antibody synthesis and the immune response in vivo. Since mixtures of antibodies have different properties than the individual antibodies (depending on which ones are mixed), it seems likely that the immune response should result in mixtures of compatible antibodies. Thus, the maturation of the affinity of antisera [Karush, E., (1978) Comprehensive Immunology 5. Immunoglobulins pp. 85, Plenum Medical Book Co., New York] may not only be due to the fact that a high affinity clone has been stimulated but that an antibody has been produced which can enhance the affinity of another antibody already present in the serum.

Several types of antibody mixtures have been examined. Combining antibody B101 with antibody A102 results in the expected dose-response curve in that the logit plot shows the inhibition line for the mixture to be between the inhibition curves for the separate antibodies. B101 and A102 cannot bind hCG simultaneously. With antibodies B101 and B103, which can bind to the hormone at the same time, the logit plot for the mixture follows the plot for the high affinity antibody at low antigen cncentrations and approaches the line for the low affinity antibody at high antigen concentrations. The mixture of antibodies B101 and B102 is the major result of this work in that the affinity of the mixture is higher than either antibody. As shown in Table I, this result is reproducible and statistically significant.

Once the presence of a cooperative effect had been established, the question of mechanism arose. There was a loss of enhancement when the F(ab) fragment was substituted for the antibody. This result indicates two important characterstics of the cooperativity. First, a larger fragment of the antibody than F(ab) is required. Second, the possibility that the binding of one antibody changes the conformation of the hormone thus leading to a higher affinity of the second antibody (an allosteric model) became unlikely. Another possible explanation for the enhancement is that it is caused by intermolecular crosslinking of bivalent antibodies and antigen (as in formation of a circular complex consisting of two antibody and two antigen molecules, which Schumaker, et al. [Schumaker, V. N., et al., (1973) Immunochemistry 10:521] have predicted to be very stable complexes). The results with $F(ab')_2$ indicate that, at the very least, this cannot be the sole mechanism. Alternatively, interaction of the F(c) regions in a complex is consistent with all data from the liquid phase radioimmunoassays. The solid phase assays seem to indicate that F(c) interactions are not the only factor since the antibodies are already anchored to the plastic and enhancement still occurs. Of course, the mechanism may be different for the two types of assays. Finally, the results with antibodies B101 and B103, which can bind simultaneously to hCG but do not have a cooperative interacton, indicate that idiotype-anti-idiotype interactions may play a role (i.e., B101 and B102 may have an idiotype-anti-idiotype interaction between the free F(ab) arms left after binding to hCG while B101 and B103 are not related in this way). It should be emphasized, however, that the fact that B101 and B103 are not enhancing argues very strongly that some characteristic of the individual antibody (such as spatial orientation of the epitope or the nature of the idiotope) is extremely important in the cooperative effect. Further experiments are necessary for complete characterization of the mechanism.

The possibility that mixtures of antibodies may have higher affinity than the individual antibodies has two major implications. First, an opportunity for increasing the affinity of monoclonal antibodies is provided. Second, the capacity to mix a series of antibodies in a defined system makes possible a more complete understanding of the subtle effects which occur in a polyclonal immune sera.

Extent of Synergistic Interactions

By performing radioimmunoassays with all paired combinations of five monoclonal antibodies, the prevalence of synergistic interactions was measured. The following table shows the pairs of antibodies that are synergistic (have a higher affinity for antigen when mixed) measured by double antibody radioimmunoassays. A plus sign indicates that the antibody identified at the top of the column is synergistic with the antibody named at the left. A minus sign indicates no synergistic interaction.

Antibody	A103	B101	B102	B103
A102	–	–	+	+
A103		–	+	+
B101			+	–
B102				–

Thus, out of ten possible pairs, five are synergistic. Actually, the proportion is much higher because some of these antibodies cannot bind antigen simultaneously under any conditions. If these pairs are eliminated, then 5 out of 7 pairs are synergistic.

Increase in Specificity of Mixtures of Monoclonal Antibodies

FIG. 7 shows the logit inhibition curves of a mixture of B101 and B102, antibody B101, and antibody B102 with hCG (the antigen) and hLH (a hormone with a very similar structure). Distinguishing between these two hormones is very important in the diagnosis of pregnancy and cancer. Graph A shows that the mixture of B101 and B102 can bind hCG about 170 fold stronger than hLH. Graph B shows that B101 can bind hCG about 50 fold stronger than hLH. Graph C shows that B102 is more sensitive to hCG than hLH by about 10 fold. Therefore, the mixture of antibodies is more specific than the individual antibodies.

In order to further characterize those monoclonal antibodies which when mixed provide enhanced affinity for antigen, further studies were undertaken. These studies showed that monoclonal antibodies possessing this property could be differentiated from those which do not, based upon ability to form a stable complex between the mixture of antibodies and the antigen. This complex appeared as an extra band in gel electrophoresis.

More specifically, 1–5 μg hCG and 1–5 μg antibody mixture were placed in 11 cm long tubes of 3, 4, 5, 6 and 7 percent polyacrylamide gels. The tubes were then subjected to a constant current of 4 milliamps per tube. The method used is essentially the same as that described for the second dimension in O'Farrell, P. H. (1975) J. Biol. Chem. 250: 4007–4021, except that there is no SDS. After separation, the gels are stained with a suitable dye.

The appearance of an extra band based upon the formation of a complex between the monoclonal antibody mixture and antigen indicates that use of such monoclonal antibody mixtures in assays for the appropriate antigen will provide enhanced sensitivity.

In addition to electrophoresis, antibody combinations resulting in enhanced affinity were recognized by an extra high molecular weight peak on gel filtration chomatography. More specifically, 1–5 μg hCG containing a suitable amount of radioiodinated hCG (0.1–10.0 μCi) were mixed with 1 to 10 micrograms of the antibody mixture and chomatographed in 0.015M Hepes buffer, pH 7.4–0.9% NaCl—1 μg/ml BSA over a 1 cm diameter—24 cm long Sephacryl S-400 column. Mixtures of B101+B102+hCG giving enhanced affinity gave rise to an extra peak whereas mixtures of B101+B103+hCG known not to give an increase in affinity in solution failed to produce the extra peak. Thus, this procedure is also useful in determining which pairs of antibody will be effective.

Although the invention has been described with particular reference to the use of mixtures of monoclonal antibodies in assays to provide enhanced sensitivity, it is understood that the invention also embraces use of the mixtures to provide enhanced specificity and such other purposes as may subsequently be discovered, including perhaps in vivo treatment methods for various conditions.

What is claimed is:

1. An immunoassay for an antigen providing enhanced sensitivity which comprises contacting the antigen under suitable conditions with effective assaying amounts of each of at least two monoclonal antibodies which bind to different antigenic sites on the antigen and which are capable under appropriate conditions in a totally liquid phase of forming a stable complex which includes the antigen and all of the monoclonal antibodies, the monoclonal antibodies being further characterized in that they form a stable complex with the antigen when subjected to gel electrophoresis in the presence of the antigen and when subjected to gel filtration chromatography in the presence of the antigen, and detecting the complex which results from contacting the antigen with the monoclonal antibodies.

2. An immunoassay according to claim 1 which comprises two monoclonal antibodies.

3. An immunoassay according to claim 1, wherein said antigen is a polypeptide and said antigenic sites are amino acid sequences contained therein.

4. An immunoassay according to claim 1, wherein said polypeptide antigen is human chorionic gonadotropin.

5. An immunoassay according to claim 1, wherein said polypeptide antigen is follicle stimulating hormone.

6. An immunoassay according to claim 1, wherein said polypeptide antigen is thyroid stimulating hormone.

7. An immunoassay according to claim 1, wherein said polypeptide antigen is luteinizing hormone.

8. An immunoassay according to claim 1, wherein said effective assaying amounts are amounts of each monoclonal antibody sufficient to permit at least about 10 percent of said antigen present in said sample to form said stable complex.

9. An immunoassay according to claim 1, wherein the amounts of each antibody relative to the amount of other antibodies is in the range from about 10^6:1 to about 1:10^6.

10. An immunoassay according to claim 9, wherein the amount is in the range from about 10^2:1 to about 1:10^2.

11. An immunoassay according to claim 9, wherein the amount of each antibody relative to the amount of other antibodies is substantially the same as the ratio of the binding constants of the antibodies to the antigen.

12. An immunoassay according to claim 4, which comprises two monoclonal antibodies, one of which binds to the α chain of human chorionic gonadotropin and the other of which binds to the β chain of human chorionic gonadotropin.

13. An immunoassay according to claim 12, wherein the antibody which binds to the α chain is designated A102 or A103 and the antibody which binds to the β chain is designated B102 to B103.

14. An immunoassay according to claim 4, which comprises two monoclonal antibodies, both of which bind to the β chain of human chorionic gonadotropin, said antibodies being designated B101 and B102.

15. An immunoassay according to claim 1, wherein at least one of the monoclonal antibodies is adsorbed onto a solid matrix.

16. An immunoassay according to claim 1, wherein the monoclonal antibodies are dissolved in a suitably buffered solution.

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